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ERRATUM

Line 4 under heading *Intestinal flora,* appearing on page 177 of The Journal of Nutrition for October, volume 51, no. 2, is misplaced and should have been line 2. The paragraph is reprinted here for removal and pasting over the 14 lines at bottom of page 177.

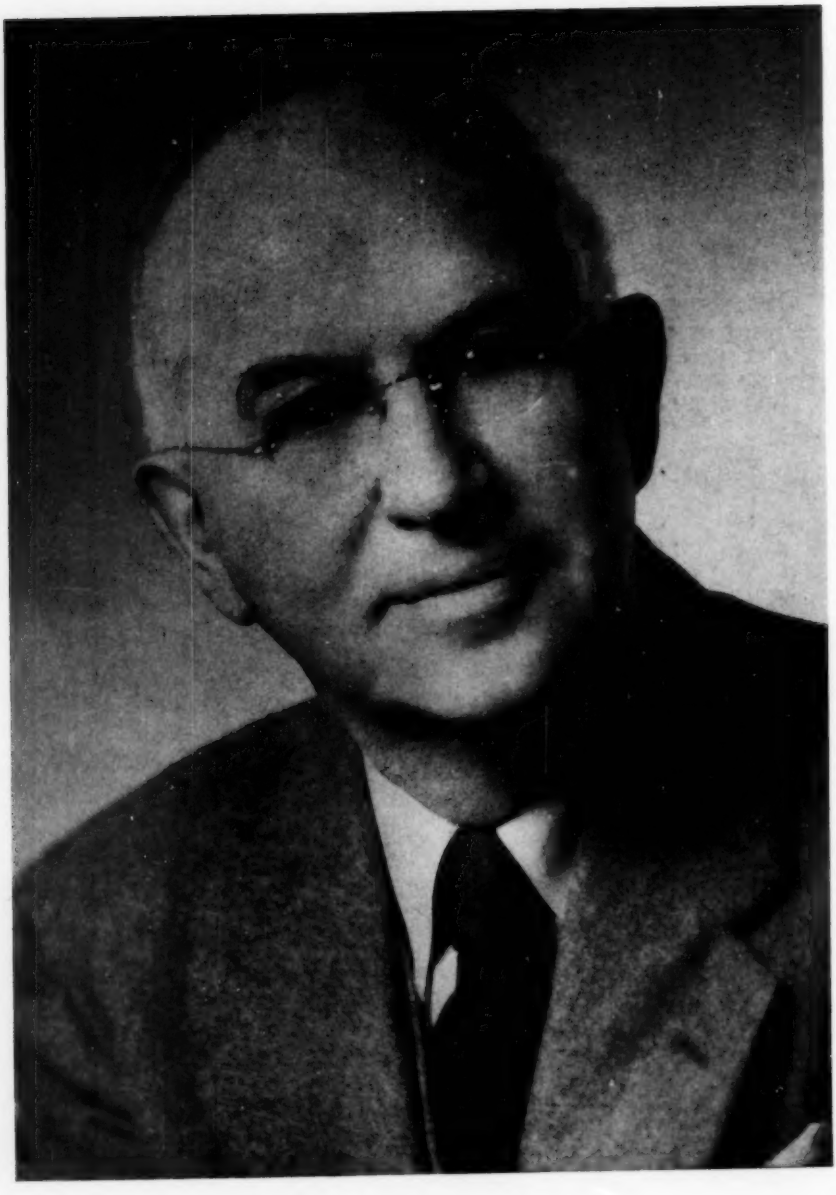
Intestinal flora

For the sake of clarity, counts of aureomycin-resistant coliforms are omitted from figures 1 and 2. Since vitamin B_{12} appeared to have no effect on the flora, counts from animals fed B_{12}-deficient and comparable B_{12}-containing diets were averaged to simplify presentation of the data.

Figure 1 indicates the microflora changes induced by aureomycin within the ileum and cecum. The bars on these graphs are arranged by pairs according to the organism counted, as indicated along the abscissa. The first bar of each pair represents the intestinal population in those rats not fed aureomycin, while the action of aureomycin is indicated by the second bar of the pair. The first step of each pair of bars shows the ileal and the second step the cecal count. Each test was made

EDWIN BRET HART

(1874 – 1953)



EDWIN BRET HART

EDWIN BRET HART

(December 25, 1874 – March 12, 1953)

Edwin Bret Hart was born near Sandusky, Ohio, on December 25, 1874, the son of William and Mary Hess Hart and the youngest of their family of 14. As a student in Sandusky High School, from which he graduated in 1892, he came in contact with Dr. E. L. Moseley, a teacher of physics and biology who first interested him in science. After high school Hart proceeded to the University of Michigan, where he studied chemistry and medicine.

While at Michigan he was an assistant to a distinguished professor of analytical chemistry, Dr. E. D. Campbell, who had lost his sight due to an explosion in the laboratory. He often told of his relationship with Professor Campbell and frequently referred to the bicycle trips they took together. They used the then common tandem bicycle, which required the same team work they enjoyed in the laboratory. This ride was a daily occurrence, and Hart admitted that after a hard day in the laboratory Campbell often supplied most of the power. His first scientific publication was with Professor Campbell and was entitled "On the Quantitative Determination of Hydrogen by Means of Palladous Chloride."

In 1897 he went to the New York State Experiment Station at Geneva where he worked with Dr. W. H. Jordan, which experience gave him a new approach to experimental work. It was undoubtedly his experience at Geneva that encouraged him to go to Germany for advanced work in the field of chemistry and biochemistry. So, at the very beginning of the century he set out for Germany to study under the famous biochemist, Albrecht Kossel, who was then at Marburg. After a year Professor Kossel moved to Heidelberg and Hart ac-

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companied him. Because of this transfer he was prevented from obtaining a higher degree. However, Professor Hart always felt that the right kinds of training and experience were as valuable as degrees. He published one paper as a result of his work in Kossel's laboratory and this experience introduced him to studies on proteins and all the intricacies of protein chemistry.

After two years in Germany he returned to Geneva, where he remained for a period of 4 years. In 1906 Dean W. A. Henry of the College of Agriculture invited him to come to the University of Wisconsin as Head of the Department of Agricultural Chemistry and Chemist in the Experiment Station. Undoubtedly one important factor which attracted him to Wisconsin was the fact that Dr. Stephen M. Babcock, who was there, had been at the Geneva Experiment Station from 1882 to 1888 and Hart had become acquainted with his work.

At the New York State Experiment Station Hart became interested in two areas of research: the study of chemical changes in cheese during ripening and the importance of phosphorus compounds in plants and animals. With L. L. Van Slyke he published a number of papers dealing with enzymes in cheese, proteolytic compounds formed during the ripening of cheese and the relation of gasses and salts to chemical changes taking place in the ripening process.

This early work brought him in contact with milk and dairy products and he continued his interest in this subject during his entire life. Shortly after coming to Wisconsin he published a simple method for the estimation of casein in cow's milk and studied the variations in the amount of casein in milks. His interest in this was undoubtedly dependent on the fact that 15 years earlier Doctor Babcock had developed a method for the estimation of fat in milk. Hart believed that the market price of milk should be based on the protein content as well as the percentage of fat. It is interesting to reflect that if as much attention had been given to the Hart casein test as was given to the Babcock fat test, the dairy industry would today encounter less difficulty in coping with surplus

butterfat and the high price of butterfat as compared to the other constituents of milk. In other words, in those early days greater emphasis might have been placed on the nutritional value of milk below the cream line.

In 1903 Professor Hart published, with W. H. Andrews of the Geneva Experiment Station, a classical bulletin dealing with the status of phosphorus in food materials. This work clearly differentiated between the so-called bound or organic phosphorus and free or inorganic phosphorus. The following year Patten and Hart studied the principal phosphorus compounds in wheat bran and established the importance of what we know today as phytin. His interest in phosphorus continued after coming to Wisconsin and one of his first contributions from the Agricultural Chemistry Laboratory at Wisconsin was a paper by McCollum and Hart on "The Occurrence of a Phytin Splitting Enzyme in Animal Tissues." In 1909 Hart and McCollum, in cooperation with Professor J. G. Fuller, established the role of inorganic phosphorus in the nutrition of animals. As a result of these studies McCollum concluded in 1910 that all of the phosphorus needed by an animal for skeleton, nuclein or phosphatide formation can be drawn from inorganic phosphate. This early work is especially significant in the light of the tremendous developments that have taken place during the past 40 years relating to phosphorylated organic compounds. Professor Hart pioneered in these early studies.

Up until this time no specific emphasis had been placed on nutrition as such. Professor Hart had been interested in analytical chemistry, in dairy chemistry, and in proteins, enzymes and mineral elements, but he was now to pioneer in the new field of nutrition. A few years before Hart came to Wisconsin, Doctor Babcock had initiated experiments with growing heifers on restricted rations. The results of these experiments had not been conclusive and when Hart arrived in 1906 Doctor Babcock presented the results of his preliminary work and suggested to Dean Henry that a more comprehensive experimental plan should be inaugurated. The

results of these studies with rations from single plant sources led to the publication of Research Bulletin No. 17, which has become a classical report since it laid the foundation for most modern research in nutrition.

It was about this time that limited federal funds were being made available for the support of research in Agricultural Experiment Stations. To be sure, the workers in those days were plagued with requests for reports of experimental results, as are our modern workers. In order to be able to show progress in these reports, Hart outlined two projects, namely, Adams No. 8, which dealt with mineral metabolism and the mineral requirements of animals, and Adams No. 10, which dealt with vitamins and other organic nutrients in animal nutrition. This approach was typical of Hart's philosophy — namely, to have a broad research program, one which was not confining but which allowed the investigator to modify his plan as new ideas became available.

No one individual has contributed more to research on minerals and vitamins in nutrition than Professor Hart. He was instrumental in the discovery that iodine is important in preventing goiter in animals and as a result of this discovery the livestock industry has been saved millions of dollars. He was an enthusiastic advocate of iodized salt for humans in order to prevent simple goiter in children, and served on committees which attempted to promote legislation which would make the iodization of salt compulsory. Later he and associates developed a method for the stabilization of iodine in salt which would insure the maintenance of a standard iodine content.

In 1923 he initiated a comprehensive study on the relation of iron to nutrition, and in cooperation with other members of the Department clearly demonstrated, in 1928, that iron cannot be utilized for building hemoglobin unless it is accompanied by a small amount of copper. This discovery was presented by Professor Hart at the Federation Meetings in Ann Arbor, Michigan, in the spring of 1928. After his presentation he was given a standing ovation by the members. Such

recognition has been given to only a few members of the American Society for Biological Chemists.

The demonstration that inorganic iron can be used for hemoglobin production only when it is accompanied by small amounts of copper was not only of importance in human nutrition but had real value in the prevention of anemia in farm animals, especially pigs. While Hart was pleased to see another discovery made in his laboratory put to immediate practical use, he was also interested in the basic aspects of this discovery and gave much attention to the mechanism by which copper functions in the living cell. Further studies on copper led him into studies of other trace elements such as manganese, zinc, cobalt and boron. He and his co-workers demonstrated that both manganese and zinc have important functions in the animal body. Extensive attempts were made to demonstrate that boron was essential in animal nutrition, but it was impossible to produce a diet low enough in boron to produce any ill effect in the animal. It was recognized, of course, that boron is an important element in plant nutrition. He also studied the importance of cobalt in preventing anemia in cows and sheep before its relationship to vitamin B_{12} was demonstrated.

He was one of the first workers to show the toxic effect of fluorine in farm animals and these early results impressed him with the deleterious effects of this element even when used in small amounts as a therapeutic agent. Extended investigations were undertaken under his direction in 1928 to study the physiological effects of fluorine at various levels upon farm and laboratory animals. This study was brought about because rock phosphate was being introduced as an economical source of calcium and phosphorus in practical animal feeding. The results showed that rock phosphate was highly unsatisfactory as a mineral supplement because of the harmful effects of the fluorine present. As a result of these studies improvements were made in the production of rock phosphate so that a large portion of the fluorine was eliminated.

Later when the use of fluorine in drinking water was suggested as a means of controlling dental caries, Professor Hart was frequently called upon as an advisor and consultant in this program. As late as January, 1952, he testified before the House Select Committee to Investigate the Use of Chemicals in Foods and Cosmetics (82nd Congress) on the possible deleterious effects of fluorine. His advice at this time was typical; namely, that more extensive studies should be carried out before far-reaching conclusions could be reached. Perhaps he was overly cautious in opposing the rapid application of this procedure, but his opposition was based on sound knowledge of the dangers inherent in such a program.

He was frequently called upon for advice, but his response was always the same — secure the needed facts through research. In fact, it was a question of this kind which led him to demonstrate that urea could be used as a source of nitrogen in ruminants. An industrial concern came to him with experimental data that had been reported in Germany indicating that simple forms of nitrogen might be used as a replacement for part of the protein in the diet. He searched the literature and found that every bit of evidence indicated that this was not true. However, he was not satisfied with the available facts, and went to the dairy barns to get a few cows in order to set up an experiment on urea. He soon demonstrated that under proper conditions urea can be used by ruminants, and as a result of these studies urea is used in a practical way today.

Professor Hart's interest in minerals and their relation to nutrition also led him into the field of food technology. In the manufacture of condensed milk the sterilization process involves heating the milk at a fairly high temperature for a considerable length of time. During this sterilization process the milk often coagulates, and even if the coagulum is broken up by shaking, the product is not accepted by the consuming public. Hart, together with Dr. H. H. Sommer, showed that the main factor in the heat coagulation of milk is dependent upon the composition of milk salts. They showed that this

difficulty could be remedied on a commercial scale by the addition of the proper amount of sodium citrate or disodium phosphate.

Although Hart worked in the vitamin field most of his life, his achievements present a peculiar anomaly. It is doubtful that we can associate Hart's name with the discovery of a specific vitamin or the isolation of a new vitamin. In this respect his experiences paralleled those of Sir Jack Drummond, who, though active in the vitamin field for more than a quarter of a century, has no vitamin linked to his name. Such are the unpredictable rewards of fickle fortune. However, he had a hand in studies on every one of the known vitamins. It is told that he was instrumental in helping Doctor McCollum differentiate vitamin A from vitamin B because he felt that McCollum was spending too much money for the purchase of the sugar — lactose. In the early studies McCollum used as part of the ration for his experimental animals the milk sugar lactose, but it was rather expensive and Professor Hart suggested that he use a cheaper form of carbohydrate. When this was done the ration was not only deficient in vitamin A but also in the water-soluble vitamin complex. The reason for this was the fact that lactose as manufactured in those early days carried with it considerable quantities of the B vitamins which were present in the original milk. Hart was associated with Steenbock and others in studies on the chemical properties of the antiscorbutic vitamin or vitamin C. He was associated with Steenbock in the early work on vitamin A and especially on the antirachitic vitamin, which Doctor Steenbock later showed could be produced by exposure of foods to ultraviolet light. His interest in vitamin D was stimulated largely by the difficulty which poultry raisers were encountering in attempting to produce chicks for broilers through indoor feeding. When they were reared indoors the chicks did not grow and their legs became deformed. The condition was known as leg weakness. This deficiency was shown to be due to a lack of vitamin D and as a result of this work the broiler industry was saved.

He was associated with many of the studies dealing with the B vitamins. In most cases other members of his staff took the initiative in these studies, but he was genuinely interested in the progress of each study. Nothing thrilled him more than to see a new fact thoroughly established and he was pleased when members of his staff made these new and fundamental discoveries. He was always helpful to the many students who frequently came to him for advice. Often he went to the literature to find helpful hints for investigators, regardless of whether they were working directly with him or with some of his colleagues. A total of 46 students received their Ph.D. degrees under Professor Hart, but he was helpful to at least three times this number of students.

Professor Hart's pleasing voice and affable manner made him an effective public speaker both to scientific and lay audiences. He was frequently called upon to give public addresses but he turned down a very large percentage of the invitations. He realized that he could not give a good lecture without proper preparation and he never failed to take the necessary time for such preparation. Whenever he did accept a speaking engagement, he would immediately proceed to work on his manuscript. Often he would have his paper ready months before the time of delivery.

He had the ability to present the most difficult results in simple and understandable words. He tried to instill this ability in his students and was often critical when students handed him reports which were poorly prepared.

He was a member of the American Society of Biological Chemists and a charter member of the American Institute of Nutrition. He also held membership in the American Association for the Advancement of Science, the American Chemical Society, the Society for Animal Production and the American Dairy Association. He was a member of Alpha Chi Sigma, Alpha Zeta, Phi Lambda Upsilon, Sigma Xi and Phi Kappa Phi and an honorary member of Phi Beta Kappa.

He received several professional awards and honors, among which were the Borden award from the American Dairy Asso-

ciation in 1941 and the honorary degree of Doctor of Science from the University of Wisconsin in 1949. He was elected to the National Academy of Sciences in 1944, an honor which he highly treasured, but unfortunately he never attended a meeting of the Academy. He did not enjoy meetings and when he did go to national meetings one or two baseball games were usually included in the itinerary. His failure to attend scientific meetings was unfortunate because he had much to contribute and many would have liked to become better acquainted with him.

On May 15, 1931, the members of his Department gave a dinner in his honor and presented him with bound volumes of his papers. This was given in commemoration of his 25 years of service as leader in research and as chairman of the Department of Agricultural Chemistry. At the time of his official retirement in 1945 his friends and colleagues gave him another dinner, which was attended by several hundred people.

While Professor Hart did not like honors or special recognition, it is interesting that he went out of his way to pay special tribute to Doctor Babcock, who preceded him. A few years ago when the Babcock award was given by the food technologists in Chicago he agreed to talk on the life of Doctor Babcock. A few weeks before the scheduled program he was stricken with a very severe case of arthritis. In spite of this great physical handicap he was determined to proceed to Chicago to give his paper. It was only after much persuasion that he agreed to have someone else present the paper which he had written.

At this point it is interesting to relate the final outcome of this attack of arthritis. It was in May, 1950, that Hart began to suffer from sciatica. He consulted orthopedic surgeons, physical therapists and many others, but there was no improvement during a period of three months. X rays indicated the presence of lime deposits in the lower lumbar region as the cause of the severe pain. Hart, therefore, went back to his early experiments with animals and wondered if

it would be possible to remove the calcium deposits by producing an acidosis. In other words, he made himself an experimental guinea pig. Instead of using hydrochloric acid, he decided to use ammonium chloride because a larger quantity of chlorine could be ingested through the use of this compound. He took 4 gm of the ammonium chloride daily, two after the morning meal and two after the evening meal. He followed this treatment for three weeks and at the end of this period he found that the pain had left and he could walk normally; there was no recurrence of the trouble. He realized that his own case was complicated by the use of diathermy and massage during the period that he took ammonium chloride. However, his improvement was much greater during the period of ammonium chloride treatment than at any other stage. This stimulated his interest in the entire field of arthritis and during the last few years he gave much of his time to study and research on this problem. Many of his friends, and total strangers, wrote to him for the procedure which he used. Several individuals who tried his treatment encountered distinct improvement, while others, of course, failed to find much relief.

He was an outstanding teacher. As a lecturer he was pleasing, clear and stimulating. He was a skillful and searching quiz master, immediately detecting superficial knowledge and impatient of it. In addition to the course in dairy chemistry mentioned earlier, he gave the general or elementary course in agricultural chemistry, later biochemistry.

When a younger member of the staff took over his elementary course, Professor Hart agreed, after many requests, to give an advanced course in nutrition. Students from all over the campus took this course and the discussions included many areas outside the field of nutrition. He would completely lose himself during the discussions and often the class would not be dismissed until 30 or 45 minutes after the scheduled time for it to close. In addition to his formal teaching he conducted a general seminar which was equally stimulating to everyone who sat in it.

Besides his research and teaching he was a successful administrator. He took over as Chairman of the Department when he arrived in 1906 and continued until 1944. As an administrator he worked hard to keep his staff intact. There were few losses of personnel from his Department over the 38 years during which he served as its head. He expected much from his associates but he never attempted to direct their work or to indicate what problems they should study. He was anxious to cooperate with workers in other departments and encouraged his staff to do the same. In fact, it was his successful cooperation with the workers in many departments that has done much to break down departmental barriers at the University of Wisconsin.

In 1903 Professor Hart married Annie Virginia DeMille. For many years the Harts, together with their one daughter, Margaret, lived within two blocks of the laboratory. This location was very convenient, since he visited the laboratory on Sundays as well as on weekdays. For many summers the Hart family proceeded to the tip of Door County in Wisconsin, where they had a cottage on Green Bay just outside the village of Ellison Bay. Professor Hart enjoyed sailing on the bay and swimming, as well as playing tennis on the court adjacent to his cabin. Mrs. Hart died June 28, 1936, but Margaret, together with her husband, Professor Russell Larson, lived with him in the family home. During more recent years he and his daughter and son-in-law enjoyed Christmas trips to Mexico, Central America, South America and Hawaii. He was fond of sports, both as spectator and participant. He skated and played tennis even in the later years of life, but made his sports recreation and not work.

On March 11, 1953, Professor Hart came to the laboratory as usual. Although he had been officially retired for almost 8 years, he visited the laboratory daily and continued his interest in all activities in the Department. He left the laboratory about 4 o'clock in the afternoon and spent an unusually pleasant evening with his family at home. During the night

he was stricken with a coronary attack and died on March 12, 1953.

If one were to write a complete story of Professor Hart's life, it would involve writing a complete history of nutrition during the first 50 years of this century. However, much of his work can be traced in papers which have appeared in the Journal of Nutrition. His life was exceptionally rich in outstanding achievements and great leadership and in kindly inspiration to those who had the good fortune of knowing him.

C. A. Elvehjem

EDITORIAL REVIEW

TOTAL DIGESTIBLE NUTRIENTS AS A MEASURE OF FEED ENERGY

L. A. MAYNARD[1]
Cornell University, Ithaca, New York

(Received for publication May 14, 1953)

Some years ago the writer ('44) presented a review of the Atwater system of calculating the caloric value of diets which revealed some misunderstandings and misuse of the system. In studying the papers in question the origin of a present-day misconception as to just what total digestible nutrients (TDN) actually measure in evaluating feeds for animals became apparent. Later the writer ('47) called attention to the fact that TDN does not mean exactly what the term implies, because the long-used method of calculating this takes account of certain urinary losses as well as of undigested nutrients. A lack of appreciation of this fact continues to be responsible for certain errors in the use of TDN in experimental work and in calculating digestible energy. Thus, it seems worthwhile to make clear the basis of these errors by reviewing the development of the TDN measure.

The present method of calculating TDN arose from combining calorie values for human foods with data for digestible nutrients in cattle. Atwater (1874–1875) published an extensive report on European investigations with cattle which brought German feeding standards to the attention of American workers for the first time. He described Wolff's standard,

[1] The writer is indebted to Dr. L. E. Harris, Utah State College, for reviewing some of the papers here cited, while he was on the staff of Cornell University.

which was based on digestible protein, digestible fat and digestible carbohydrates, and on his "albumenoid ratio," which was later called "nutritive ratio" in our standards. Some 15 years later, Atwater (1890) cited Rubner's factors for calculating the potential energy value of nutrients — namely, 4.1 Cal. per gram for carbohydrates, 4.1 for protein, and 9.3 for fat — and proposed their use in developing feeding standards for cattle. These factors for carbohydrate and fat were gross calorie values, but the one for protein was corrected for the portion of the gross energy which was lost in urine. Atwater pointed out that these same factors could be applied to the digestible nutrients of cattle feeds, assuming that the calculation of urine energy loss which Rubner had shown to hold for dogs and man applied to cattle. Atwater set forth "available fuel values" for cattle feeds on this basis and proposed a feeding standard accordingly. Later he developed, on the same basis, his physiological fuel values for human foods and diets, which are in use today. Atwater's proposal for cattle was not followed by others, probably because, as stated by Henry (1898), "while it is important to study, from a scientific basis, the fuel value of rations, for practice a statement of the several nutrients themselves is more explicit and satisfactory." Nevertheless, Atwater's proposal must have had an influence, perhaps unrecognized, on the thinking of later workers, because the present-day calculation of total digestible nutrients takes account of digestion losses and a calculated urine loss in accordance with the Rubner factors, although arrived at quite differently from Atwater's method.

This development came gradually. The Wolff standards as modified by Lehmann in 1897 appeared in American textbooks and bulletins. The Wolff-Lehmann tables contained a "sum of nutrients" resulting from adding together the digestible protein, fat and carbohydrate.

Hills (1900) discussed both the Atwater and the Wolff-Lehmann standards and included a new feature in the latter. He added together the digestible carbohydrates and fat, after multiplying the latter by 2.25 ($9.3 \div 4.1$), and listed this sum

instead of fat and carbohydrate separately. He pointed out that this was a simplification based on the long-accepted calculation of the second term of the nutritive ratio, a calculation which Wolff had devised to express fat as "carbohydrate equivalent" on a gross calorie basis. This combination of digestible carbohydrate and fat was followed by others. It was not until 1910, however, that the final step was taken. In that year both Hills and associates ('10) and Woll and Humphrey ('10) added in digestible protein also, and called the sum "total digestible nutrients" or "total digestible matter." Standards were thus set forth in terms of digestible protein and digestible nutrients. This method was later adopted by Henry and Morrison ('15). These various authors referred to this final step as a simplification, but beyond that no explanation was made. Thus arose the formula which has been in use since that time:

digestible protein + digestible carbohydrate + digestible fat ($\times$ 2.25) = TDN. (1)

If it was intended to summate the digestible nutrients on an equivalent carbohydrate basis, in terms of gross calorie value, as was done for fat by Rubner and later by Hills, then protein should have been multiplied by the factor 1.36 (5.65 $\div$ 4.1), since protein has a gross value of 5.65. It seems likely that the relationship represented by the Rubner factors, which Atwater had used to calculate his available fuel value, was responsible for the calculation as actually made. Here protein has the same caloric value as carbohydrate (4.1) because calculated urine losses have been deducted. Perhaps it was intended to arrive at a value, expressed in pounds of nutrients, comparable to Atwater's values, which took account of urine as well as fecal losses. But the term "total digestible nutrients" implies that digestion losses only are taken into account, and this seems to be the commonly accepted meaning today. According to this meaning, the correct formula should be:

digestible protein ($\times$ 1.36) + digestible carbohydrate + digestible fat ($\times$ 2.25) = TDN. (2)

The use of the present formula (1) disregards over 25% of the energy of the digested protein on the basis that it is lost in the urine. This is a matter of greater significance for high than for low protein feeds, as is brought out by the following data calculated on the basis of figures in the appendix, table 1, of Morrison ('48):

	TDN BY CURRENT FORMULA	TDN BY REVISED FORMULA
	lb.	*lb.*
Corn, dent, grade 2	80.1	81.3
Corn gluten feed	76.0	79.5
Cottonseed meal, 45% protein and over	78.4	84.8

The differences involved in the two methods of calculation have also been discussed in the recent text by Abrams ('50).

The differences cannot be considered to be of practical importance in connection with established feeding standards because the requirements have been determined on the basis of the TDN values calculated by the current formula and, thus, the two sets of data fit together. In fact, TDN as now calculated is a better measure of feeding value, in the sense that it disregards protein energy which is lost in the urine anyway. But it is not very satisfying scientifically to eliminate a loss based on human experiments which does not apply to herbivora, particularly since the ratio of energy to nitrogen in the urine, on which the Rubner factor for protein is based, does not hold for herbivora. The urine of the latter contains much more energy per unit of nitrogen because of the relatively large amount of hippuric acid present, as shown originally by Kellner and associates (1896) and discussed in more detail by Armsby ('08).

The literature contains several comparisons of TDN vs. metabolizable energy as measures of feeding value. Metabolizable energy is arrived at by subtracting, from the total energy intake, the energy lost in the feces, urine and intestinal gases. Since the method used for calculating TDN takes account of certain of the same urine losses, as previously

explained, the comparisons would have greater significance if TDN were arrived at by the revised formula (2), which takes account of digestion losses only. The same is true for comparisons of TDN and net energy measures. TDN is a shifting base for any calculation or comparison because the extent of the error in assuming that the energy-nitrogen ratio in the urine of man holds for herbivora depends on the proportion of nitrogen excreted as hippuric acid. This proportion is greater for roughages than for concentrates. Even if it is felt that none of the considerations discussed in this paragraph has practical importance, the use of the correct procedures is clearly preferable scientifically.

Another error which developed from the false assumption that the long-used formula for arriving at TDN (1) takes account of digestion losses only, is the use of the factor, 1814, for converting pounds TDN into calories. The writer has not been able to trace with certainty the origin of this factor. The literature, and specifically the report by Brody and coworkers ('34), indicates that it originated 30 or more years ago from the following calculation:

$$4 \text{ Cal. per gram} \times 453.6 \text{ gm per pound} = 1814. \qquad (3)$$

The factor, 4 Cal. per gram was Atwater's physiological fuel value for carbohydrate. The original objective of the use of 1814 may have been to calculate a value which would take account of urine as well as fecal losses, a measure comparable to Atwater's available energy for man. The limitation of such a calculation for cattle, because of the differences in the character of the urine losses, is obvious from the previous discussion. In any event, the actual use that has been made of the factor 1814 during the past 20 years has been for the calculation of digestible energy. This use assumes that the digestible protein as well as digestible fat is expressed on a carbohydrate equivalent basis in the TDN formula, which is not true, as previously explained.

To calculate the energy represented by the digested nutrients expressed in pounds, the correct procedure would be to

obtain TDN by the revised formula previously listed (2), which gives digestible protein its true calorie value, and then to multiply by grams per pound. Alternatively, the pounds of digestible carbohydrates, protein and fat can be multiplied by their respective gross calorie values per pound and the products added together. By either method the calculated digestible energy in the cottonseed meal listed on page 18 is approximately 1,670 Cal. per pound of the feed, compared with a value of 1,538 Cal. arrived at by the factor 1814. The under-valuation caused by the use of the latter factor is less for low protein feeds, but it results in the case of all.

Schneider ('47) has published an extensive study of the composition and digestibility of feeds used throughout the world. In the case of nearly 500 of the experiments reviewed, data on both TDN and digestible energy were presented. From a study of these data, the details of which are being prepared for publication, Schneider [2] has concluded that the approximate average factor for Cal. per gram TDN is 4.38, instead of the factor 4 used in arriving at the figure of 1,814 Cal. per pound. On this basis the factor for calculating digestible energy per pound from pounds TDN becomes 1987. Experimental support is thus given for the writer's conclusion that the use of the factor 1814 substantially undervalues the caloric equivalent of TDN. While any single factor can apply only approximately to various feeds and rations, the value proposed by Schneider rests on a sound experimental basis and its use in place of 1814, where an average factor seems adequate for the purpose, is clearly to be recommended. The longer procedure outlined in the preceding paragraph should have a distinct advantage in connection with certain types of experimental work.

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FOLIC ACID DEFICIENCY IN THE GUINEA PIG [1]

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INTRODUCTION

The production of experimental nutritional deficiencies in the guinea pig has become practicable with increasing knowledge of the dietary requirements of this species. Mannering ('49) has reviewed the recent progress in this field. Growth in young guinea pigs equivalent to that achieved when presumably complete pelleted rations were fed has been reported by Roine, Booth, Elvehjem and Hart ('49) and by Reid ('53) using diets consisting of purified materials.

PGA (pteroylglutamic acid, folic acid) was found to be an essential nutrient for the guinea pig by Woolley and Sprince ('45). In the absence of this vitamin, weanling animals developed growth failure and succumbed in about three weeks. Innes, Innes and Moore ('49) produced leukopenia, anemia and relative immaturity of both myeloid and erythroid precursors in the bone marrow of mature guinea pigs by the prolonged subcutaneous administration of PGA antagonists. Since these hematologic changes were similar to those found by Spicer, Daft, Sebrell and Ashburn ('42) in rats fed purified diets containing insoluble sulfonamides, it appeared worthwhile to attempt to induce folic acid deficiency in the guinea pig by sulfasuxidine (succinylsulfathiazole) feeding.

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[2] Markle Scholar in Medical Science.

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METHODS

Guinea pigs of the Connaught strain were purchased from a dealer [4] and arrived at the laboratory when they were about 6 weeks old and weighed approximately 250 gm. They were fed a commercial pelleted diet [5] for 7 to 10 days. During the next week a transition to the experimental diets was made by including an increasing amount of the purified diet. The animals were divided among the various diets in a random manner. The ratio between the sexes was kept as constant as possible. The average weight at the beginning of the experimental period ranged between 270 and 320 gm. Weights were determined three times weekly and the final weight was obtained after 6 weeks on the purified diet. The rate of gain in grams per day was calculated.

The composition of the purified diet is given in tables 1 and 2. The dry ingredients were mixed mechanically in large amounts and the oily constituents and 10% water added to weighed portions of the dry base as needed. Each pig received an oral supplement of 5 mg of ascorbic acid in a freshly prepared water solution administered by pipette 6 times weekly. The animals were housed individually in wire-bottomed cages in a room maintained at a constant temperature (76°F.) and 50% relative humidity.

The hematologic observations were made at the end of the 6-week experimental period, using capillary blood obtained from the ear or from heart blood. No difference was found between the two sampling methods. Counts were made in the usual manner, using Bureau of Standards certified pipettes. Hemoglobin was determined as oxyhemoglobin (Evelyn, '36). The packed cell volume was determined by the micro method of Guest and Siler ('34) as modified by Guest and Weichselbaum, or by that of Wintrobe ('51) when sufficient heart blood was available. The corpuscular constants were calculated according to the method of Wintrobe. Bone marrow aspirations were performed with a 20-gauge short bevel lumbar puncture

[4] Carworth Farms.

[5] Rockland Guinea Pig Diet.

TABLE 1

Composition of purified diet[1]

CONSTITUENTS	AMOUNT
	%
"Vitamin-free" casein	30.0
"Alphacel"	20.0
Sucrose	38.2
Salt mixture (H.M.W.)[2]	4.0
Potassium acetate	2.5
Magnesium chloride	0.5
Sodium chloride	0.5
Choline	0.3
Cottonseed oil (fortified with vitamins A, D, and E)	4.0

[1] Vitamin supplements (mg per kg): Thiamine HCl 18, riboflavin 24, niacin 300, pyridoxine HCl 18, calcium pantothenate 90, *p*-aminobenzoic acid 100, biotin 1, PGA 3, vitamin B_{12} 0.05, inositol 1,000, 2-methyl-1,4-naphthoquinone 3. Vitamin A 19,500 I.U., vitamin E 20 I.U., vitamin D 2,400 I.U. per kilogram, derived from natural concentrates.

[2] Hubbell, R. B., L. B. Mendel and A. J. Wakeman. A new salt mixture for use in experimental diets. J. Nutrition, *14:* 273, 1937.

TABLE 2

Mineral composition of diet

MINERALS	PURIFIED DIET (calculated)	ROCKLAND DIET (Mfgr.'s analysis)
	%	%
Total ash	7.78	7.64
Calcium	0.87	1.40
Phosphorus	0.44	0.53
Magnesium	0.34	0.30
Potassium	1.36	1.62
Sodium	0.32	0.68
Chlorine	0.72	0.71
Manganese	0.0005	0.006
Iron	0.021	0.044
Copper	0.0014	0.0003
Iodine	0.0002	0.003
Zinc	0.000	0.0025
Ca: P	2.0	2.6
Ca: Mg	2.5	4.7
Na: K	0.23	0.42

needle inserted into the distal end of the femur. Repeated aspirations in the same animal at frequent intervals were possible.

RESULTS

These observations were made over a period of one year on 8 separate groups of animals. No invalidating differences attributable to sex or to differences between these groups were found upon statistical analysis, and the results have been combined. Half the animals on each of the experimental diets did not receive vitamin B_{12}. Since statistical analysis failed to detect effects associated with the presence or absence of this vitamin, this variable has been omitted from the tables.

Forty-two animals were fed the commercial diet supplemented with 5.0 mg of ascorbic acid 6 times weekly. Growth on this diet was superior to that observed on other diets in use in this laboratory (table 3). Both a commercial dog food [6] supplemented with cabbage and a widely used scorbutigenic diet supplemented with 5 mg of ascorbic acid daily (Woodruff, Cherrington, Stockell and Darby, '49) proved to be poor diets for guinea pigs. When ascorbic acid was omitted from the purified diet, scurvy occurred in approximately three weeks almost simultaneously in all the animals in the group and was surprisingly uniform in severity, as determined by gross pathological examination. The incorporation of 0.02% aureomycin hydrochloride in the complete purified ration resulted in a rapid weight loss during the week of transition and three of the 9 pigs in this group died. The growth rate of the survivors did not appear to be influenced by the aureomycin.

Experiments designed to define the role of PGA in the nutrition of the guinea pig consisted of comparisons between 5 pairs of diets designated A to E. The results are presented in tables 3 to 8. The performance of the animals on the complete purified diet was chosen as a common reference point. The omission of PGA from this diet (A-PGA) depressed the growth rate significantly, but no hematologic disturbances were found. In series B, 1% sulfasuxidine was added to the

[6] Purina Dog Chow.

diet. In the absence of PGA growth was inhibited and some of the animals died before the end of the experimental period. Anemia, leukopenia and changes in the bone marrow consisting of a pronounced shift toward immaturity in the erythroid

TABLE 3

Growth rates on various diets during a 6-week experimental period

DIET	PABA	SULFA	RATE OF GAIN	
		%	*gm/day ± S.E.M.*	
Scorbutigenic diet[1] plus ascorbic acid			1.10 ± 0.78 (8)[2]	
Purina dog chow plus cabbage			0.98 ± 0.41 (8)	
Complete purified diet plus 0.02% aureomycin HCl			3.76 ± 0.47 (6)[2]	
Purified diet without ascorbic acid			−3.44 ± 0.82 (5)[4]	
Rockland			4.88 ± 0.18 (42)***[5,6]	
A	+	0	3.82 ± 0.17 (50)	2.74 ± 0.21 (35)***[5,7]
B	+	1	3.80 ± 0.47 (9)	−0.35 ± 0.28 (23)***
C	−	0	3.40 ± 0.29 (12)	−0.73 ± 0.60 (11)***
D	−	1	3.03 ± 0.84 (3)	−1.28 ± 0.39 (3)***
E	+	1	2.12 ± 0.39 (15)***	0.30 ± 0.35 (14)***

[1] Woodruff et al. ('49).

[2] Number of animals given in parentheses.

[3] Average for the survivors only.

[4] Survived only 21 days.

[5] Significance of differences from series A (+ PGA) is indicated by asteriaks: *Pr. < 0.05; **Pr. < 0.01; ***Pr. < 0.001.

[6] With PGA.

[7] Without PGA.

series, with the presence of increased numbers of large pronormoblasts, were found. No typical megaloblasts were seen on the smears. The peripheral blood showed a relative agranulocytosis in the presence of severe leukopenia, and in many animals anisocytosis and Howell-Jolly bodies were strik-

TABLE 4

Average white blood counts at end of experimental period

SERIES	PABA	SULFA	PGA PRESENT	PGA ABSENT
		%	*Cells/mm³ ± S.E.M.*	
Rockland			7,950 ± 450 (27)[1]	
A	+	0	6,530 ± 470 (35)	5,420 ± 490 (29)
B	+	1	5,690 ± 910 (9)	3,090 ± 490 (22)***[2]
C	—	0	8,670 ± 1570 (12)	4,170 ± 1136 (10)*
D	—	1	8,030 ± 700 (3)	1,630 ± 940 (3)**
E	+	1	6,670 ± 710 (15)	2,090 ± 380 (14)***

[1] Number of animals given in parentheses.

[2] Significance of differences from series A (+ PGA) is indicated by asterisks: *Pr. < 0.05; **Pr. < 0.01; ***Pr. < 0.001.

TABLE 5

Average hemoglobin concentration at end of experimental period

SERIES	PABA	SULFA	PGA PRESENT	PGA ABSENT
		%	*gm/100 ml ± S.E.M.*	
Rockland			13.95 ± 0.14 (27)[1]	
A	+	0	14.26 ± 0.25 (35)	13.54 ± 0.34 (27)
B	+	1	13.85 ± 0.44 (8)	11.94 ± 0.62 (19)***[2]
C	—	0	14.16 ± 0.30 (12)	12.15 ± 0.41 (10)***
D	—	1	12.93 ± 0.21 (3)	9.27 ± 2.52 (3)***
E	+	1	13.83 ± 0.46 (11)	11.07 ± 0.63 (11)***

[1] Number of animals given in parentheses.

[2] Significance of differences from series A (+ PGA) is indicated by asterisks: *Pr. < 0.05; **Pr. < 0.01; ***Pr. < 0.001.

TABLE 6

Average packed cell volume at end of experimental period

SERIES	PABA	SULFA	PGA PRESENT	PGA ABSENT
		%	% ± *S.E.M.*	
Rockland			45.1 ± 0.6 (18)[1]	
A	+	0	43.0 ± 1.0 (16)	44.8 ± 1.2 (13)
B	+	1	43.5 ± 1.4 (8)	36.8 ± 2.3 (14)**[2]
C	—	0	45.8 ± 0.9 (12)	39.8 ± 1.3 (10)
D	—	1	42.7 ± 0.3 (3)	27.7 ± 7.3 (3)***
E	+	1	44.6 ± 1.4 (11)	35.6 ± 2.3 (8)**

[1] Number of animals given in parentheses.

[2] Significance of differences from series A (+ PGA) is indicated by asterisks: *Pr. < 0.05; **Pr. < 0.01; ***Pr. < 0.001.

ing. PGA in the amounts present in the complete diet prevented these changes (B + PGA).

The omission of para-aminobenzoic acid (PABA) rather than the inclusion of sulfasuxidine characterized the diets in

TABLE 7

Average red blood count at end of experimental period

SERIES	PABA	SULFA	PGA PRESENT	PGA ABSENT
		%	*Cells* × 10^6/mm^3 ± *S.E.M.*	
Rockland			4.42 ± 0.10 (18)[1]	
A	+	0	4.44 ± 0.13 (16)	3.93 ± 0.20 (15)*
B	+	1	3.22 ± 0.20 (15)***[2]	3.67 ± 0.30 (18)*
C	—	0	4.64 ± 0.10 (12)	4.19 ± 0.13 (10)
D	—	1	3.97 ± 0.09 (3)	3.21 ± 0.81 (3)*
E	+	1	3.85 ± 0.16 (11)**	3.63 ± 0.17 (11)***

[1] Number of animals given in parentheses.

[2] Significance of differences from series A (+ PGA) is indicated by asterisks: *Pr. < 0.05; **Pr. < 0.01; ***Pr. < 0.001.

TABLE 8

Average mean cell volumes at end of experimental period

SERIES	PABA	SULFA	PGA PRESENT	PGA ABSENT
		%	μ^3 ± *S.E.M.*	
Rockland			103 ± 3 (18)[1]	
A	+	0	98 ± 2 (16)	107 ± 3 (13)**
B	+	1	112 ± 5 (8)**[2]	111 ± 4 (14)**
C	—	0	99 ± 1 (12)	95 ± 3 (10)
D	—	1	108 ± 2 (3)*	89 ± 14 (3)
E	+	1	117 ± 3 (11)***[3]	95 ± 2 (8)

[1] Number of animals given in parentheses.

[2] Significance of differences from series A (+ PGA) is indicated by asterisks: *Pr. < 0.05; **Pr. < 0.01; ***Pr. < 0.001.

[3] Only value significantly higher (Pr. < 0.05) than that found on the Rockland Diet.

series C. In the presence of PGA (C + PGA), growth and hematologic values did not differ from those found in the animals on the complete diet (A + PGA). When PGA was omitted (C — PGA), growth failure occurred and hematologic

changes similar to those found on diets containing sulfonamide but no PGA were observed. Three of the 11 animals in this group failed to develop either growth failure or changes in the hemopoietic system. This increased the variability and diminished the probability of significant differences between some of the averages. In series D, both methods of producing the deficiency picture were combined. The number of animals used was too small to allow conclusions concerning the increased severity of the changes, but it is clear that PGA was effective in preventing them. In series E the PGA was given subcutaneously in doses of 100 μg three times weekly. Five micrograms of vitamin B_{12} weekly were given by subcutaneous injection to one-half of these animals. The PGA solution used was irritating to the tissues of the abdominal wall, and large areas of hemorrhage and fibrosis were noted at the injection site in almost all of the animals, with peritonitis occurring twice. The poor growth on diet E + PGA may be attributable to this trauma. No control injections were given to the E — PGA group, which developed changes essentially the same as those found in the other group receiving sulfonamides (B — PGA).

The mean corpuscular volume and red blood count (tables 7 and 8) followed a different pattern from that of the leukocyte count, packed cell volume and hemoglobin concentration. The lower erythrocyte counts and higher mean corpuscular volumes appeared to be associated with sulfonamide ingestion, but also occurred in the A — PGA group. Only in the PGA-deficient groups receiving sulfonamide and demonstrating anemia was the depression of the erythrocyte count highly significant (diets B — PGA and E — PGA). Comparison of the mean corpuscular volume of the animals in the experimental groups with that found in the animals receiving the commercial diet reveals that only group E + PGA showed a statistically significant macrocytosis. The magnitude of these differences is not great enough to warrant interpretation without further study.

DISCUSSION

Although the growth rate of guinea pigs on the purified diet used in these experiments was significantly less than that achieved on a commercial pelleted diet, it appeared to be satisfactory for nutritional experiments. Other workers (Roine et al., '49; Reid, '53) have used animals but a few days old for their studies and have observed growth rates of almost 7.0 gm per day on both pelleted and purified diets. Our older animals grew at a slower rate.

These observations have permitted a study of the growth rates achieved on a scorbutigenic diet adequately supplemented with ascorbic acid. The poor growth observed on such a diet has suggested to us that it is not adequate for the guinea pig. Scurvy appeared regularly on the deficient purified diet and the gross pathological lesions were quite similar in extent. This purified diet should be useful in studies of experimental scurvy, especially those involving biochemical estimations.

The anemia, leukopenia and relative immaturity of the erythroid precursors in the bone marrow noted in the deficient animals could be prevented in every instance by the inclusion in the diet of 3.0 mg/kg of folic acid. These changes must be interpreted as representing folic acid deficiency in the guinea pig. They are similar to the hematologic picture of the folic acid-deficient rat (Spicer et al., '42), monkey (Day, '44) and that found in human anemias which respond to folic acid administration (Jukes, '52). Since death usually occurs in sulfonamide-treated animals before the anemia has become severe and soon after the appearance of the marrow changes, these experiments should be interpreted as representing an acute deficiency of this vitamin. Kodicek and Carpenter ('50) have demonstrated that macrocytosis will occur in the sulfasuxidine-treated rat if amounts of folic acid sufficient for survival but insufficient to permit hematologic remission are administered at intervals. Unfortunately these authors did not study the bone marrow of their animals. Girdwood ('51) was able to produce a severe anemia in 500-gm guinea pigs,

with the appearance of cells in the marrow suggesting megaloblastic change, by feeding 2% sulfasuxidine to animals already rendered anemic by aminopterin administration. Anemia did not occur in 5 weeks in his animals following sulfasuxidine feeding alone. Similar experiments have not yet been performed on guinea pigs fed purified diets. However, the induction of such a chronic deficiency might be expected to produce a picture of greater immaturity.

We have not attempted to determine the minimum requirement for folic acid. The level fed (3.0 mg per kilogram of diet) is adequate to prevent hematologic changes for at least 8 weeks.

The importance of para-aminobenzoic acid in the nutrition of the guinea pig has been studied. The quantity of this factor (100 mg per kilogram) present in the diet prevented the occurrence of hematologic changes when folic acid was withheld, whereas absence of both PGA and PABA from the diet resulted, in a majority of the animals, in a deficiency state similar to that induced by sulfasuxidine. Diets lacking only in PABA produced growth rates and hemograms that could not be differentiated from those found when PABA was included in the diet. Consequently, the PABA requirement of the guinea pig can be met by adequate PGA, and it may be that PABA is not an essential nutrient for the guinea pig except as a precursor of folic acid.

The production of folic acid deficiency in guinea pigs either by means of sulfasuxidine feeding or by excluding both PABA and folic acid from the diet, and the prevention of this deficiency in each case by folic acid alone, further substantiate this hypothesis. The site of action of sulfasuxidine and consequently that of PABA is probably in the intestine. Therefore, we have assumed that when the diet is deficient in folic acid, the intestinal flora of the guinea pig is unable to synthesize adequate folic acid unless PABA is present in the diet. Conversely, it may be assumed that this synthesis is inhibited by sulfonamides. The failure of three animals to develop the deficiency on diets devoid of both PABA and folic acid sug-

gests that variability in the intestinal flora may be important in this synthesis.

SUMMARY

1. These studies confirm the need of the guinea pig for a dietary source of folic acid. No dietary requirement for PABA by this species could be demonstrated, but PABA was found partially to replace PGA in the diet of the guinea pig.

2. PGA deficiency in the immature guinea pig was characterized by growth failure, leukopenia, anemia and immaturity of the erythroid precursors in the bone marrow.

3. This deficiency could be produced either by the omission of PABA from a purified diet deficient in PGA or by the inclusion of both PABA and 1% sulfasuxidine.

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USE OF INDICATORS IN THE MEASUREMENT OF THE DRY MATTER INTAKE OF GRAZING ANIMALS[1]

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TWO FIGURES

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INTRODUCTION

In order to measure the amount of dry matter consumed by grazing animals, it is necessary to determine the indigestibility of the ingested herbage and the quantity of feces voided per unit of time. Under grazing conditions, the use of indicator methods to effect these measurements provides problems which are much different from those encountered in the study of feedstuffs which are readily sampled and of which the quantities consumed are known. With grazing conditions, the indicator employed to measure digestibility must be a natural constituent of plants. The selective grazing tendency of animals precludes the manual sampling of herbage for chemical analysis (Hardison and Reid, '52). This, obviously, would not be a problem if the indicator used and the nutrients were uniformly distributed throughout the plant. These problems, bearing upon the measurement of the digestibility of *grazed* herbage, are circumvented by the method proposed by Reid et al. ('52). Later work by Hardison and Reid ('52) demonstrated that, also with random sampling of feces, this method

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(Reid et al., '52) provides a satisfactory estimate of digestibility.

The feces produced may be secured by the use of collection bags and harnesses, but this apparatus may influence grazing behavior and is not readily adapted to use with grazing female animals. It appears that "artificial" indicators fed in known quantity may be used to estimate the amount of feces voided per unit of time. Consequently, a combination of indicators, one occurring naturally in the forage and employed as an indicator of indigestibility and another which is fed to the animal in known quantity and employed as an index of the total quantity of feces voided per unit of time, may accomplish, simultaneously, the measurement of digestibility and the rate of consumption of herbage by grazing animals.

Coup ('50) examined the value of Cr_2O_3 as an indicator of the feces output by grazing cows. Excellent agreement between the measured (by bag) and estimated outputs was obtained when Cr_2O_3 was determined in representative (total collection) feces samples. Coup found that the amount of feces produced could also be estimated accurately from "grab" samples of feces taken in the morning and evening and bulked over a 14-day period. The data on the output of feces obtained by Coup were employed by Percival ('50) in combination with indigestibility coefficients derived from fecal nitrogen excretion rates to estimate the dry matter intake of grazing cows.

The high potential value of a method employing a combination of two indicators for the measurement of dry matter intake requires that further attention be given to the problem. From the standpoint of the practical application, such a method should permit accurate estimates of forage intake to be derived from the analysis of "grab" samples of feces obtained at convenient times for periods of one or more days. The object of these experiments was to determine the accuracy with which the intake of herbage by grazing animals could be measured, employing the chromogen procedure (Reid et al., '52) to measure indigestibility and Cr_2O_3 to determine the

fecal output when several fecal-sampling procedures were employed.

EXPERIMENTAL PROCEDURE

A series of 6 trials was conducted during a two-year period to determine the accuracy with which Cr_2O_3 serves as an indicator of the quantity of feces voided by steers, hand-fed and grazing several pasture herbages.

In all of the trials, one group of three steers was fed forage clipped from the same source as, though from an area adjacent to, that grazed at the same time by a similar group of three animals. A preliminary feeding or grazing period of at least 15 days preceded the fecal collection periods. The animals used consisted of both dairy- and beef-type steers, ranging in weight from approximately 320 to 950 lb.

The botanical composition of the forages employed in these studies varied considerably from trial to trial. A mixture consisting mainly of timothy, quack grass and alfalfa was used in trials 1, 2 and 3. In trials 4 and 5 a relatively pure sward of brome grass was used, while in trial 6 a relatively pure sward of alfalfa was employed.

The hand-fed steers received daily two meals of whole, clipped herbage. The clipped herbage was fed within approximately two hours after harvesting. This allowed sufficient time to determine the moisture content of the herbage by toluene distillation. A quantity of green herbage providing a constant amount of dry matter per meal was allotted each hand-fed animal. Each hand-fed animal was administered by mouth 10 gm of Cr_2O_3 in a gelatin capsule [3] daily at approximately 7 : 00 A.M. (approximately one hour following the morning feeding). The Cr_2O_3-containing capsules were given to the grazing animals at approximately 7 : 30 A.M. Chromium oxide was administered during a period of 7 or more days (generally 10 days) prior to the beginning of, and was continued during,

[3] Through the courtesy of Dr. Sereck Fox, the Cr_2O_3-containing capsules were prepared by the Gelatin Products Division of the Schering Corporation, Detroit, Michigan. Analysis of these capsules showed them to contain 10.000 ± 0.006 gm of Cr_2O_3.

the fecal collection period. The accuracy with which fecal production was estimated with the use of Cr_2O_3 was based upon comparisons with the amounts of feces measured by bag collection.

The major feature of this study was the manner and time of sampling feces. For ease of discussion these trials have been numbered consecutively one through 6. In trials 1 and 2, total collections of feces were made during a 4-day period. In trial 3, samples of feces weighing approximately 300 gm (wet) were taken each morning and evening of a 4-day period. Since these samples were taken from the mixed contents of the collection bags, they were considered to be representative portions of feces yielded during the previous 11- or 13-hour period. The collection bags were removed at 6:00 A.M. and 5:00 P.M. Total collection of the feces also was made during this trial. Trial 4 was conducted in a manner similar to that of trials 1 and 2 except that the fecal collection period extended over a 6-day period. In trial 5, samples of feces weighing approximately 300 gm (wet) were taken each morning (6:00 A.M.) and afternoon (4:00 P.M.) from the top of the feces in the collection bags. These samples were assumed to represent the feces yielded in the last defecation prior to sampling. It is possible that the last defecation occurred as much as three hours prior to the sampling time. In trial 6, "grab" samples of feces were taken rectally from each of three hand-fed and three grazing steers at various times of the day over an 11-day period. Total collections of feces also were made during this period.

RESULTS AND DISCUSSION

In these experiments, two feeding treatments (hand feeding and grazing) were imposed. The herbage hand-fed to one group of steers was clipped from the same source as, though from an area adjacent to, that grazed by the other group of animals. The dry matter intakes estimated from the combined use of the Cr_2O_3 and plant chromogen indicators were compared, in the case of the hand-feeding trials, to the meas-

ured intakes and, in the case of the grazing trials, to the intake determined from the total collection of feces by bag and the indigestibility determined by the fecal-chromogen method. The dry matter intake determined in this way for grazing animals was considered to be the "measured" intake. The summary shown in table 1 demonstrates that the dry matter

TABLE 1

Comparison of intakes derived from the analysis of representative feces voided during periods of 4 to 11 days with the measured intakes

DRY MATTER INTAKE (LB./DAY)					
Hand-fed			Grazing		
Animal no.	Measured	Estimated	Animal no.	Measured	Estimated
Trial 1					
7	12.7	12.2	10	6.9	6.7
8	10.5	11.0	11	13.6	14.1
9	9.7	9.9	12	7.6	7.7
Trial 2					
7	14.9	15.6	10	12.5	12.3
8	13.3	14.2	11	12.0	11.9
9	12.7	11.3	12	7.7	8.6
Trial 3					
7	16.3	17.2	10	15.5	(20.4)[1]
8	14.2	12.7	11	14.0	14.6
9	12.5	13.8	12	13.1	11.9
Trial 4					
11	4.4	4.3	23	7.2	6.6
12	4.0	4.1	24	7.5	7.4
13	4.4	4.3	25	9.0	9.0
Trial 5					
1	7.2	7.8	23	11.4	11.0
2	6.6	6.4	24	8.9	8.7
3	7.4	7.2	25	11.2	11.6
Trial 6					
11	7.5	6.6	14	11.4	11.0
12	8.6	7.8	15	9.6	9.7
13	8.7	8.1	16	17.1	17.2
Mean	9.75 ± .90	9.69 ± .95		10.90 ± .72	10.59 ± .72

[1] High intake estimate for steer 10 is the result of his ingesting less than the complete dosage of Cr_2O_3; the data for this animal were excluded in computing the mean estimated intake for grazing steers.

intake of both hand-fed and grazing animals was estimated quite accurately using the two indicators when total collections of feces were made during periods of 4 to 11 days. The amounts of the two indicators were determined in feces samples representative of those obtained by total collection. The lack of agreement between the intake values for steer 10 in trial 3 resulted from his failure to retain the full dosage of Cr_2O_3 administered. This emphasizes a major source of mechanical error in the application of the technique, which, of course, may be avoided by exercising extreme care in the administration of the Cr_2O_3-containing capsules.

The coefficient of correlation between the measured and estimated intakes of dry matter was 0.981 and the relationship between the two kinds of intake values is expressed by the equation: $Y = 0.928X + 0.827$, where: Y = measured intake (pounds/day) and X = estimated intake (pounds/day). The standard error of estimate of the daily intake of dry matter determined from the use of the indicators was 0.67 lb. in these trials in which the mean measured intake was 10.2 lb. per day.

A comparison was made of the intakes estimated from the analysis of feces voided during one-half-day periods with those measured during the 4-day period of trial 3. The data shown in table 2 indicate that the intake estimated from half-day samples of feces was not as reliable as that secured by analysis of composite samples of feces representing the outgo during 4 consecutive days. It will be observed that the influence of the incomplete dosage of Cr_2O_3 to steer 10 was exerted largely in the intake estimated for one half-day period. Regardless of feeding treatment, intake estimates based upon feces voided during the day generally were higher than those derived from feces produced during the night.

Although Cr_2O_3 measured in feces representative of those voided during periods of 4 or more days was found to provide an adequate index of fecal outgo, the use of this kind of feces sample necessarily requires total collection. Consequently, the advantage of using Cr_2O_3 in the estimation of intake under these sampling conditions is automatically voided.

However, if Cr_2O_3 were excreted in the feces either uniformly or in a predictable manner, it would be possible to supplant the total collection of feces by a series of "grab" samples taken a few times during one or more days. This would obviate the necessity for fecal collection bags, allow greater use of female animals and reduce the cost of experimentation.

TABLE 2

Comparison of intakes derived from the analysis of fecal samples voided during half-day periods and a 4-day period with the measured intakes

DATE	HALF-DAY SAMPLING PERIOD	ESTIMATED DRY MATTER INTAKE (LB./DAY)					
		Hand-fed steers			Grazing-steers		
		7	8	9	10	11	12
8/ 8	day	19.1	17.0	16.2	16.9	16.5	15.2
8/ 9	night	16.6	15.2	7.9	22.2	10.7	9.9
8/ 9	day	17.0	15.2	20.9	39.6	19.0	15.9
8/10	night	15.8	12.1	17.6	19.6	12.1	10.3
8/10	day	16.6	14.2	15.6	17.2	18.9	16.4
8/11	night	16.0	15.2	12.2	12.8	11.2	11.4
8/11	day	15.0	13.4	15.0	(560.8)[2]	19.4	16.7
8/12	night	13.5	6.4	13.2	14.0	17.8	11.8
Average intakes based upon analysis of half-daily samples of feces		16.5 ± 0.57[1]	13.6 ± 1.15	14.8 ± 1.36	20.3 ± 3.43	15.7 ± 1.32	13.4 ± 1.01
Estimated intakes based upon analysis of 4-day composite sample of feces		17.2	12.7	13.8	20.4	14.6	11.9
Measured intakes		16.3	14.2	12.5	15.5	14.0	13.1

[1] Standard error of the mean.

[2] Value for steer 10 on day 8/11 was excluded from average.

Further experiments were conducted with a view to the refinement of the fecal sampling procedure. In trial 5, samples were taken from the top of the feces in the collection bags at approximately 6:00 A.M. and 4:00 P.M. Although these were not true "grab" samples, they were assumed to be representative of the feces yielded in the last defecation prior to sampling. Intake estimates based upon the analysis of partial col-

lections of feces and upon those of samples representative of the feces produced during 4 consecutive days are compared in table 3 with the average measured daily dry matter intakes. The intakes estimated from the samples taken in the morning and afternoon were more uniform for the hand-fed steers than for those grazing. A uniformly higher intake by grazing

TABLE 3

Comparison of intakes derived from the analysis of fecal samples voided during the morning and afternoon and a 4-day period with the measured intakes

DATE	SAMPLING TIME	ESTIMATED DRY MATTER INTAKE (LB./DAY)					
		Hand-fed steers			Grazing steers		
		1	2	3	23	24	25
8/2	P.M.	7.0	6.3	7.0	14.9	15.8	38.2
8/3	A.M.	8.4	6.0	6.2	8.6	6.4	10.4
8/3	P.M.	7.6	6.3	7.5	10.0	6.6	14.1
8/4	A.M.	7.0	5.2	6.4	9.5	5.7	8.7
8/4	P.M.	7.4	7.4	9.0	10.7	5.6	25.4
8/5	A.M.	7.4	6.4	6.5	9.3	8.0	10.2
8/5	P.M.	7.0	6.5	7.5	23.7	30.2	17.2
8/6	A.M.	6.9	6.3	7.2	10.6	...	9.8
8/6	P.M.	..	..	..	..	13.4	..
8/7	A.M.	..	..	..	..	8.6	..
Average intakes based on analysis of partial samples		7.3 ± 0.18 [1]	6.3 ± 0.21	7.2 ± 0.32	12.2 ± 1.48	11.1 ± 2.66	16.8 ± 3.63
Estimated intakes based on analysis of 4-day composite sample		7.8	6.4	7.2	11.0	8.7	11.6
Measured intakes		7.2	6.6	7.4	11.4	8.9	11.2

[1] Standard error of the mean.

steers was estimated from feces sampled in the afternoon than from those sampled in the morning. It would appear that the time of day when herbage was consumed and the amounts consumed at various times were considerably different for the two groups of steers. It is to be expected that, as a reflection of variable grazing habits, the degree of dilution of Cr_2O_3 in the feces would vary, depending upon the intake of the

animal. (The data obtained in a subsequent experiment suggest that the feces samples taken during trial 5, particularly the afternoon samples, were voided quite some time prior to the removal of the bags.)

Since the results of the first 5 trials indicated that the measurement of Cr_2O_3 in feces representative of those produced during 4 or more days gave satisfactory intake estimates, and

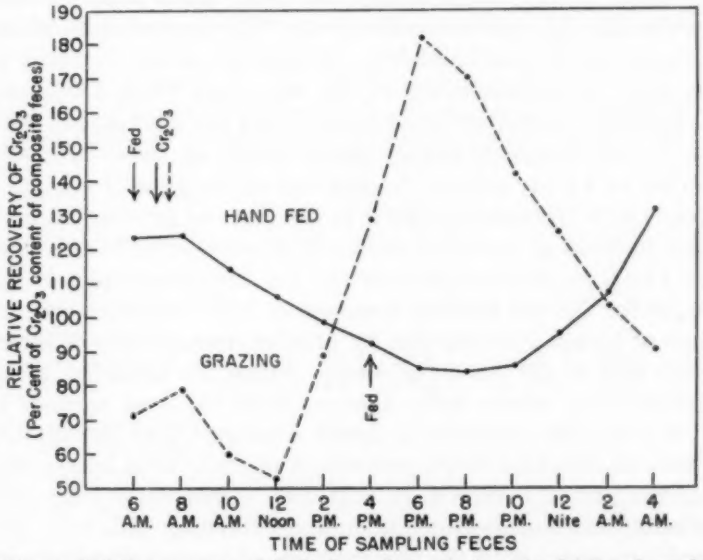

Fig. 1 Relative recovery of Cr_2O_3 from feces of steers hand-fed and grazing pasture herbage.

that the excretion of Cr_2O_3 per unit of feces dry matter varied (particularly under grazing conditions) with time of day, a further experiment was conducted. During the 11-day period of trial 6, feces samples were taken rectally at two-, 6-, 8-, 10-, or 12-hour intervals during each of two or more 24-hour periods. From this fecal sampling procedure, composite patterns of Cr_2O_3 excretion during a 24-hour period by both hand-fed and grazing animals were determined and are shown in figure 1. Expression of the amount of Cr_2O_3 in the "grab"

samples of feces as a percentage of the amount of Cr_2O_3 in feces compounded from total collection during the entire period allows the Cr_2O_3 excretion rates of animals consuming different quantities of forage to be compared. Since the Cr_2O_3 excretion-time patterns were similar for all animals on one feeding system (hand feeding or grazing), these were compounded to form the patterns of Cr_2O_3 excretion obtained under hand-feeding and grazing conditions. Each point on both curves (fig. 1) represents from 6 to 33 observations. These patterns show that hand-fed and grazing steers excreted in the feces at certain hours of the day from 80 to 130% and 50 to 180%, respectively, as much Cr_2O_3 per unit of dry matter as was found in feces representative of those produced during an 11-day period. In addition to the greater variation from 100% recovery of Cr_2O_3 in the feces of grazing animals than in those of hand-fed steers, it is interesting to note that the Cr_2O_3 excretion-time patterns are almost completely opposite for the two feeding treatments. This indicates that the time of forage consumption by grazing animals was different from that of the hand-fed steers. Since the hand-fed group received two meals daily (one at 6:00 A.M. and another at 4:00 P.M.), the patterns in figure 1 suggest that the grazing steers consumed a large proportion of their total intake during the period 6:00 P.M. to 8:00 A.M. Apparently very little grazing was done between noon and 6:00 P.M. This is in general agreement with the observations of the grazing behavior of cattle made by Castle et al. ('50), Hancock ('50) and Seath and Miller ('46).

Table 4 summarizes a comparison of the average measured intakes of dry matter during the 11-day period with the average of the intake estimates based upon the analysis of "grab" samples of feces and with the estimated intakes derived from the Cr_2O_3 levels of composite samples of feces collected during the 11-day period. As evidenced by the relatively large standard errors shown in table 4 and the Cr_2O_3 excretion-time patterns in figure 1, the variable excretion of Cr_2O_3 precludes the random sampling of feces. The rates of

recovery of the ingested Cr_2O_3 from the representative feces of the grazing and hand-fed animals were 100.2 and 101.2%, respectively.

Because of the close agreement among the Cr_2O_3 excretion-time patterns of the individual animals in each treatment group, it appeared that these could be combined (as shown in fig. 1), and examined for the purpose of determining those times of the day when feces might be sampled to secure the greatest accuracy. This study showed that "grab" samples

TABLE 4

Comparison of average intakes derived from the analysis of "grab" samples of feces with those derived from the analysis of an 11-day composite sample of feces and with the measured intakes

KIND OF INTAKE	DRY MATTER INTAKE (LB./DAY)					
	Hand-fed steers			Grazing steers		
	11	12	13	14	15	16
Average intakes based on analysis of 45 "grab" samples of feces	7.2 ± 0.28[1]	7.7 ± 0.32	7.6 ± 0.25	17.3 ± 1.41	13.2 ± 1.58	17.9 ± 1.70
Average intakes based on analysis of 11-day composite sample	6.6	7.8	8.1	11.0	9.7	17.2
Measured intake	7.5	8.6	8.7	11.4	9.6	17.1

[1] Standard error of the mean.

of feces taken at 2:00 to 3:00 P.M. or 1:00 to 2:00 A.M. contained approximately the same concentration of Cr_2O_3 as was found in representative feces (obtained by total collection during 11 days) of both grazing and hand-fed steers. However, from the standpoint of animal management, these are not convenient times to sample feces. Lactating cows are generally confined in a barn for milking during the morning and afternoon; therefore, feces may be taken readily during these periods. Since the Cr_2O_3 excretion-time patterns observed appear to be constant (at least under certain grazing conditions),

it would be feasible to sample feces at any hour of the day, assuming that the rate of recovery of Cr_2O_3 is known for a particular sampling time.

Consequently, the data were examined with a view to selecting an hour of the morning and afternoon at which times the samples of feces procured would form a composite sample effecting approximately 100% recovery of the ingested Cr_2O_3. Various combinations of morning and afternoon sampling times resulted in satisfactory intake estimates. The average recoveries of Cr_2O_3 from feces compounded from wet "grab" samples taken at 6:00 A.M. and 4:00 P.M., the most convenient sampling times under our conditions, were 104% in the hand-feeding trials and 99.95% in the grazing trials. An examination was made of the accuracy with which the intake could be estimated as a result of sampling feces at 6:00 A.M. and 4:00 P.M. and forming a composite sample over periods of one to 8 days. These estimates are compared in table 5 with the measured average intakes during the 11-day period. Since the mean recovery of ingested Cr_2O_3 from the feces of hand-fed steers was 104%, the Cr_2O_3 content of the feces was corrected to a recovery of 100% to derive the estimated intakes of this group of animals (table 5). The intake estimates derived from "grab" samples obtained at these times of the day and compounded on an equal wet-weight basis over an 8-day period were in good agreement with the measured intakes. The average estimated intakes during the 8-day period were different from the measured intakes of hand-fed and grazing animals by only 2.4 and 2.4%, respectively. As would be expected, the inter-day variation in the intake of grazing animals was considerable, whereas the estimated intakes of hand-fed animals were relatively constant.

In order to save time and labor, the practical application of indicator methods requires that the compounding of feces samples be done on a wet-sample basis. It was found in this experiment that equal amounts of wet feces taken at 6:00 A.M. and 4:00 P.M. could be combined, since the dry matter contents of samples taken at these times were not significantly

different statistically. Consequently, the estimates of intake were similar whether feces were bulked on a wet or dry basis.

Although it is not known whether or not the fecal sampling procedure employed in this experiment (compounding over periods of several days the "grab" samples taken at 6:00 A.M. and 4:00 P.M.) would provide equally satisfactory results under all grazing conditions, other data obtained subsequently in this laboratory suggest that very satisfactory

TABLE 5

Comparison of intakes derived from the analysis of feces taken at 6:00 A.M. and 4:00 P.M. during periods of various length with the measured intakes

LENGTH OF SAMPLING PERIOD	DRY MATTER INTAKE (LB./DAY)					
	Hand-fed steers [1]			Grazing steers		
	11	12	13	14	15	16
days						
1	7.8	10.1	7.8	10.4	11.6	15.4
2	7.8	9.3	8.8	11.4	14.2	13.7
3	7.7	9.6	9.0	12.5	9.1	15.7
4	7.4	9.5	9.3	11.9	8.3	16.8
5	7.5	8.8	9.2	10.4	8.7	17.5
6	7.4	8.6	8.6	11.2	9.4	17.9
7	7.3	8.6	8.6	10.6	9.5	18.3
8	7.3	8.7	8.4	10.6	9.5	17.1
Measured intake	7.5	8.6	8.7	11.4	9.6	17.1

[1] Intakes of hand-fed steers corrected for Cr_2O_3 recovery of 104%.

estimates of intakes resulted from this procedure when applied under conditions greatly different from those being reported here. It seems possible that the excretion of Cr_2O_3 may be influenced by such factors as the degree of uniformity and time of forage intake, the specific gravity of Cr_2O_3, time of administering Cr_2O_3, the manner in which Cr_2O_3 is administered, plant species available for grazing, the stage of growth of herbage and such items as climatic conditions and management practices which may affect the grazing habits of animals. Also, the effects of water consumption, factors affecting the motility of the gastrointestinal tract, and the sex of the grazing animal upon the pattern of Cr_2O_3 excretion

have not been examined. The nature of the Cr_2O_3 excretion-time patterns obtained in this study (fig. 1) suggests that an influence exerted upon the concentration of the Cr_2O_3 in feces taken at 6:00 A.M. would effect a change of similar magnitude, though of opposite direction, in that of feces taken at 4:00 P.M. As a consequence, it is indicated that the concentration of Cr_2O_3 in a composite sample of feces formed from those taken at 6:00 A.M. and 4:00 P.M. may represent approximately 100% recovery under a variety of experimental conditions.

Recently, Kane et al. ('52) reported that a "diurnal" variation occurs in the excretion of Cr_2O_3 by cows consuming a ration composed of alfalfa hay, corn silage and concentrates in two meals daily. Chromium oxide was administered twice daily as a portion of the concentrate mixture. Despite differences in the sex of the animals used, the kinds of rations fed, the manner of administering Cr_2O_3 and the number of dosages of Cr_2O_3 administered daily, the Cr_2O_3-excretion patterns (fig. 2) observed in the experiments conducted by Kane et al. ('52) are remarkably similar to those (fig. 1) obtained in the present studies in which clipped herbage was hand-fed to steers. Perhaps closer agreement between the patterns would have been obtained had the times of feeding been identical. From their data, Kane et al. ('52) concluded that the excretion of Cr_2O_3 is not associated with the time of ingestion of feed. Since the Cr_2O_3 concentration of the feces taken from all cows between 1:00 P.M. and 3:00 P.M. was similar to that of the total feces yielded during a 24-hour period, these workers concluded that feces procured during this time interval resulted in accurate estimates of digestibility. However, they cautioned that the use of a single "grab" sample may cause erroneous conclusions.

It appeared to us that, should the Cr_2O_3 excretion-time patterns be constant under certain feeding conditions, it would be possible, knowing the Cr_2O_3 recovery rate at various times of the day, to compute digestibility accurately from the analysis of feces taken at any time during a 24-hour period. This

would seem to be true, particularly if the sampling were done at the same time during several days. As a consequence, a study was made of the Cr_2O_3 excretion patterns of the animals used by Kane et al. ('52). The concentrations of Cr_2O_3 in the feces shown by these workers were expressed in terms of relative recovery (fig. 2) in order that the influence of variable intakes of dry matter and Cr_2O_3 could be removed, and in order to place all data on a comparable basis. (It should

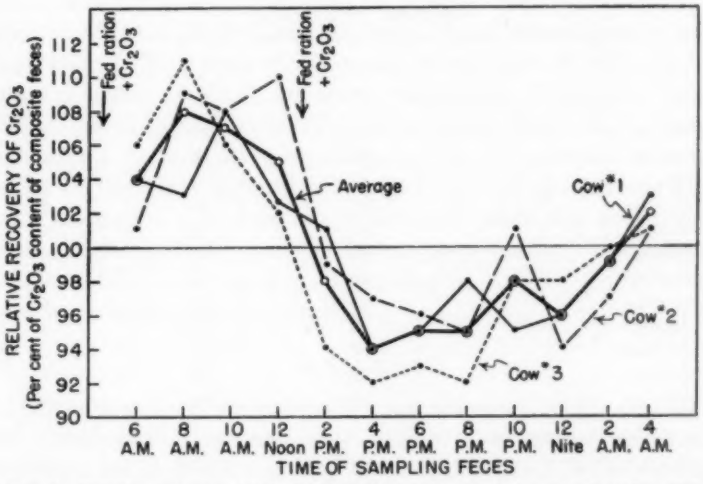

Fig. 2 Relative recovery of Cr_2O_3 from feces of cows fed a mixed ration (from data of Kane et al., '52).

be pointed out that the data employed in these computations were interpolated from the graphs presented by Kane et al. ['52] and, therefore, may not be strictly accurate.) A composite pattern was constructed from the patterns obtained with the individual cows. This pattern was considered to represent the proportion of the Cr_2O_3 in the representative feces collected during a 24-hour period which was excreted in "grab" samples of feces taken at various times during this period.

Digestion coefficients for dry matter were then computed from the fecal Cr_2O_3 values taken from the graphs presented by Kane et al. ('52) following correction for the recovery of Cr_2O_3. Correction of the Cr_2O_3 content of "grab" feces samples in this way resulted in the digestion coefficients shown in table 6. The inter-sampling time uniformity of these data

TABLE 6

Digestion coefficients computed from the data presented by Kane et al. ('52) following the correction of the Cr_2O_3 concentration of feces to represent 100% recovery

TIME	COWS		
	1	2	3
6:00 A.M.	65	64	64
8:00 A.M.	64	64	64
10:00 A.M.	65	63	64
12:00 NOON	64	66	61
2:00 P.M.	66	65	60
4:00 P.M.	65	65	62
6:00 P.M.	65	65	62
8:00 P.M.	65	65	61
10:00 P.M.	64	66	61
12:00 NIGHT	65	63	64
2:00 A.M.	65	63	63
4:00 A.M.	65	64	63
Ave. estimated coefficients based upon "grab" samples of feces	64.9 ± 0.19	64.5 ± 0.30	62.5 ± 0.41
Estimated coefficients based upon 9-day composite sample of feces	65	64	63
Coefficients determined in conventional digestion trial	64	63	63

is satisfactory. It is to be expected that the agreement between the values obtained from total collection and those from "grab" samples taken at a given time would be improved by increasing the number of days sampled.

It is concluded from the results of Kane et al. ('52) and from those obtained in the present study that the variable

excretion of Cr_2O_3 is not strictly diurnal, since the time of fed consumption and the amount of feed consumed at a given time, as well as other factors, appear to influence the Cr_2O_3 concentration-time relationship. It is possible that the time of feeding and other factors may indirectly influence the excretion pattern as a result of affecting the behavior or posture of animals. The results of both experiments indicate that the sampling of feces at two convenient times of the day (one time when the recovery of Cr_2O_3 is less than 100% and another when the recovery rate is greater than 100%) would tend to effect a predictable recovery. It is interesting to note that Coup ('50) observed a mean difference of 15% in the concentration of Cr_2O_3 between "half-daily" samples taken in the morning and evening from grazing cows. As a result of bulking, on an equal-weight basis, "grab" samples of feces taken in the morning and evening, Coup found that the concentration of Cr_2O_3 was overestimated (thus the amount of feces voided and the intake were underestimated) by approximately 3.5%. The exact times of day at which "grab" samples were taken were not stated.

SUMMARY

The adequacy of indicators for measurement of the dry matter intake was examined in a series of trials in which all herbages studied were grazed by and hand-fed to steers. Intake estimates derived from the measurement of indigestibility by the fecal-chromogen method and of fecal outgo by the use of Cr_2O_3 were similar to the measured intakes when feces representative of those voided during periods of 4 or more days were employed.

As a result of an experiment in which a detailed "grab" fecal sampling plan was employed, it was found that the Cr_2O_3 content of the feces voided at intervals of the day is extremely variable. This precludes a random sampling of feces for the purpose of estimating intake. However, the nature of the Cr_2O_3 excretion pattern with respect to time of day indicated that accurate intake estimates could be derived

from samples of feces taken at any time provided that the rate of recovery of Cr_2O_3 is known. Consequently, the adequacy of feces obtained at convenient sampling times was examined. Feces samples procured at 6:00 A.M. and 4:00 P.M. and compounded on an equal wet-weight basis during 7 or more days resulted in reliable estimates of dry matter intake.

Although the general applicability of this sampling procedure to a wide variety of grazing conditions remains to be determined, excellent results have been obtained in subsequent experiments in which conditions different from those of the present experiments were imposed. It is indicated that factors which influence the rate of Cr_2O_3 recovery from the feces taken in the morning may effect a change in the recovery of Cr_2O_3 of similar magnitude, though of opposite direction, in the feces taken during the afternoon. The mean rates of recovery of Cr_2O_3 from the feces taken from grazing steers at 6:00 A.M. and 4:00 P.M. were 71.8 and 129.3%, respectively. Wet-bulking of the feces samples taken at these two times of day resulted in an average recovery of 99.95% of the Cr_2O_3 ingested by grazing steers.

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ANTIBIOTICS, METHIONINE AND UNIDENTIFIED GROWTH FACTORS IN THE NUTRITION OF BROADBREASTED BRONZE TURKEY POULTS

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Aureomycin, streptomycin, terramycin, penicillin and 3-nitro-4 hydroxyphenylarsonic acid have been shown to stimulate the growth and improve the feed efficiency of turkey poults (Stokstad and Jukes, '50; Atkinson and Couch, '51b; Almquist and Merritt, '51; McGinnis and co-workers, '51; Patrick, '51b; Branion and Hill, '51). The growth of chicks has been shown to be improved by the addition of methionine to a ration containing only vegetable protein (Hayward and Hafner, '41; Marvel and co-workers, '44; Clandinin and co-workers, '46; Gerry, Carrick and Hauge, '48; Sunde et al., '52). Almquist ('52) points out that the poult diet should contain 0.45% methionine for maximum growth. Poult growth has been shown to be increased by adding 0.2% methionine to an all-vegetable protein diet (Bird, Marsden and Kellogg, '48). Poult growth was not stimulated by the addition of methionine to a ration containing meat and bone scraps (Johnson, '49, '50). Unidentified growth factors for the turkey poult have been reported to be present in dried skim milk, fish meal, dried whey, fish solubles, distillers' dried solubles, dried brewers' yeast, liver preparations and grass and alfalfa juices (Patrick, '51a, b; Atkinson and Couch, '51a; Scott, '51a, b, '52; Menge and Combs, '52).

The purpose of the experiments reported in the present paper was to study further the effect of supplementing an all-vegetable protein ration with methionine, distillers' dried solubles and antibiotics on the growth, feed efficiency and mortality of growing turkey poults.

MATERIALS AND METHODS

Five experiments were carried out in the laboratories of the Departments of Poultry Husbandry and Biochemistry and Nutrition of the Texas Agricultural Experiment Station, College Station. The poults used in experiments 1, 2, 4 and 5 were obtained from a commercial turkey breeder, and the hens had been fed a commercial breeder ration. The poults used in experiment 3 were from dams maintained by the Poultry Department of the Texas Agricultural Experiment Station, which were fed an all-mash breeder mash that was believed to be complete with regard to known nutrients and unidentified factor sources.

Management of the poults was the same as that reported by Atkinson and Couch ('51b).

The basal ration used in experiments 1 and 2 contained 60% soybean oil meal, 35% ground yellow corn, 2.5% steamed bone meal, 2% oyster shell and 0.5% salt. The diet was supplemented with 4,000 I.U. vitamin A, 800 I.C.U. vitamin D_3, 2 mg riboflavin, 5 mg calcium pantothenate, 12.5 mg niacin, 400 mg choline chloride, 5 μg vitamin B_{12} and 120 mg manganese sulfate per pound of feed. A coccidiostat (sulfaquinoxaline) was added to all rations. The basal ration used in experiments 3, 4 and 5 was the same as that used in experiments 1 and 2 except that the soybean level was reduced from 60% to 55% and the ground corn was replaced with ground milo at a level of 40%. This diet was calculated to contain 28.0 to 28.5% protein. A ration high in protein has been shown to be necessary for the maximum early growth of turkey poults by Lloyd, Reed and Fritz ('49) and Scott, Heuser and Norris ('48).

RESULTS

First experiment

From the results of the first experiment (table 1) it is apparent that poult growth was stimulated by supplementation of the basal diet with bacitracin (group 2), with methionine (group 3) and with methionine and bacitracin (group 4).

TABLE 1

Effect of aureomycin, bacitracin, methionine and distillers' dried solubles on the growth, feed efficiency and survival of Broadbreasted Bronze poults

GROUP NO.	SUPPLEMENTS TO BASAL DIET	BIRDS SURVIVING [1]	AVERAGE WEIGHTS, MALES AND FEMALES	FEED EFFICIENCY
			gm	*gm feed/gm gain*
	Experiment one — Duration 8 weeks			
1	None	31	1,051	2.72
2	Bacitracin, 5 mg/lb.	33	1,097	2.64
3	Methionine, 0.05%	32	1,276	2.61
4	Bacitracin, 5 mg/lb. Methionine, 0.05%	32	1,417	2.39
	Experiment two — Duration 10 weeks			
1	None	43	1,728	2.84
2	Aureomycin, 5 mg/lb.	44	1,761	2.75
3	Distillers' dried solubles, 5%	43	2,037	2.67
4	Aureomycin, 5 mg/lb. Distillers' dried solubles, 5%	48	1,975	2.29

[1] Each group contained 33 birds at beginning of test, experiment 1. Each group contained 50 birds at beginning of test, experiment 2.

When the data of this experiment were treated statistically by analysis of variance, an F value of 7.52 was obtained, whereas an F value of only 3.94 is necessary for significance at the 0.01 level of probability. It is evident, therefore, that the differences between the various treatments are real. Feed efficiency is hereby defined as "the quantity of feed required to produce a unit increase in weight." The level of bacitracin mentioned above (group 2) was 5 mg per pound. Other groups were included in this test which were fed 2.5 and 10 mg bacitra-

cin per pound of diet, respectively. The feeding of 2.5 mg bacitracin per pound failed to have any significant effect on the weight or feed efficiency of the poults. The feeding of 10 mg bacitracin per pound failed to produce results which were significantly different from those obtained from feeding 5 mg of bacitracin per pound of ration. From the results of this experiment, 5 mg of bacitracin per pound of diet might be considered a satisfactory level of the antibiotic to use in poult diets. A significant increase in the weights of the poults and an improvement in feed efficiency resulted when 0.05% DL-methionine was added to the basal diet. An additive effect on growth and feed efficiency as indicated by analysis of variance was observed when methionine was fed in the presence of bacitracin (group 4).

Second experiment

The growth of the poults in the second test was significantly increased by the substitution of 3% distillers' dried solubles for ground yellow corn and soybean oil meal (substitutions were made so as to keep the protein level constant) in the basal ration (group 3). Feed efficiency was also improved by the latter substitution (table 1). Feed efficiency was decidedly improved by the addition of aureomycin (5 mg per pound of ration) to the basal in the presence of 3% distillers' dried solubles (group 4). Growth and feed efficiency were not significantly changed when aureomycin alone was added to the basal diet under the conditions of this experiment (group 2). Treatment of the data of the second experiment by analysis of variance gave an F value of 9.11, whereas an F value of only 3.88 is required for significance at the 0.01 level of probability. The fact that aureomycin failed to increase the growth of the poults under the conditions of this experiment is not surprising, since it has been the experience of the authors in this laboratory that turkey poults do not always respond to the addition of an antibiotic to the diet.

Third experiment

Bacitracin, distillers' dried solubles and methionine each produced an increase in growth and an improvement in feed efficiency when added to the basal diet in the third experiment (table 2). When the data of this experiment were treated statistically by analysis of variance, an F value of 18.84 was obtained, whereas an F value of only 2.37 is required for

TABLE 2

Effect of methionine, penicillin, bacitracin and distillers' dried solubles on the growth and feed efficiency of Broadbreasted Bronze poults

GROUP NO.	SUPPLEMENTS TO BASAL DIET	BIRDS SURVIVING [1]	AVERAGE WEIGHTS, MALES AND FEMALES	FEED EFFICIENCY
			gm	*gm feed/gm gain*
	Experiment three — Duration 8 weeks			
1	None	43	1,390.5	2.54
2	Penicillin, 2 mg/lb.	40	1,358.6	2.32
3	Methionine, 0.05%	47	1,533.2	2.29
4	Penicillin, 2 mg/lb. Methionine, 0.05%	47	1,556.8	2.27
5	Distillers' dried solubles, 5%	49	1,743.4	2.22
6	Penicillin, 2 mg/lb. Distillers' dried solubles, 5%	49	1,822.3	2.23
7	Penicillin, 2 mg/lb. Distillers' dried solubles, 5% Methionine, 0.05%	49	1,902.7	2.14
8	Bacitracin, 5 mg/lb.	42	1,506.4	2.27
9	Bacitracin, 5 mg/lb. Methionine, 0.05%	45	1,625.3	2.29
10	Bacitracin, 5 mg/lb. Distillers' dried solubles, 5%	47	1,749.6	2.33
11	Bacitracin, 5 mg/lb. Distillers' dried solubles, 5% Methionine, 0.05%	50	1,909.2	2.16

[1] Each group contained 50 birds at beginning of the test.

significance at the 0.01 level of probability. From these data it is apparent that the differences between the various treatments are real. The results produced by the substitution of 5% distillers' dried solubles in the basal ration were particularly outstanding in this test (group 5). The addition of penicillin to the basal (group 2) failed to improve growth but did improve feed efficiency. The results produced by the addition of methionine, distillers' dried solubles and either penicillin or bacitracin were shown to be additive, when analyzed statistically by analysis of variance. Attention is directed to the almost identical results with regard to growth and feed efficiency which were produced by the use of either penicillin or bacitracin with distillers' dried solubles and methionine. It should be pointed out that penicillin was fed at the level of 2 mg and bacitracin at the level of 5 mg per pound of diet in obtaining these results. Thus evidence is provided to the effect that the two antibiotics are of equal value when fed in turkey starting diets at the levels used in this experiment.

The mortality observed in groups 1, 2 and 3 was apparently caused by the fact that the gizzards became impacted with litter which was revealed by a post-mortem examination of the dead poults. No explanation can be offered for the fact that poults in these groups appeared to be more addicted to eating litter than those in the other groups. This test was carried out during the spring, when the temperature is more favorable, and the weights of the poults at the end of the 8-week period were somewhat greater than those of the poults in the other experiments, where the birds were grown during the summer months.

Fourth experiment

Supplementation of the basal diet with penicillin (2 mg per pound) or bacitracin (5 mg per pound) produced a significant increase in growth and a decided improvement in feed efficiency in the 4th experiment (groups 2 and 5, table 3). Treatment of the data of this test by analysis of variance gave an

F value of 9.39, whereas an F value of only 3.11 is necessary for significance at the 0.01 level of probability. The substitution of 5% distillers' dried solubles in the basal diet failed to produce a significant increase in the growth of the poults

TABLE 3

Effect of penicillin, bacitracin, methionine and distillers' dried solubles on the growth, feed efficiency and survival of Broadbreasted Bronze poults

GROUP NO.	SUPPLEMENTS TO BASAL DIET	BIRDS SURVIVING [1]	AVERAGE WEIGHTS, MALES AND FEMALES	FEED EFFICIENCY
			gm	*gm feed/gm gain*
	Experiment four — Duration 8 weeks			
1	None	48	1,222	2.59
2	Penicillin, 2 mg/lb.	45	1,468	2.29
3	Distillers' dried solubles, 5%	47	1,288	2.55
4	Penicillin, 2 mg/lb. Distillers' dried solubles, 5%	48	1,498	2.24
5	Bacitracin, 5 mg/lb.	49	1,384	2.29
6	Distillers' dried solubles, 5% Bacitracin, 5 mg/lb.	49	1,413	2.25
	Experiment five — Duration 8 weeks			
1	None	37	841	2.99
2	Distillers' dried solubles, 5%	41	1,058	2.73
3	Methionine, 0.05%	40	1,133	2.69
4	Bacitracin, 5 mg/lb.	41	1,153	2.49
5	Bacitracin, 5 mg/lb. Methionine, 0.05%	43	1,302	2.43
6	Bacitracin, 5 mg/lb. Distillers' dried solubles, 5%	41	1,312	2.48
7	Bacitracin, 5 mg/lb. Methionine, 0.05% Distillers' dried solubles, 5%	44	1,439	2.30

[1] Each group contained 50 birds at beginning of test, experiment 4. Each group contained 45 birds at beginning of test, experiment 5.

(group 3) and had little effect on feed efficiency. The feeding of penicillin or bacitracin in the presence of distillers' dried solubles produced a slight additive effect on growth but had little if any effect on feed efficiency over that obtained by the addition of the antibiotics to the basal diet without the

distillers' dried solubles. It is apparent from the results of this test that the particular sample of distillers' dried solubles used contained less of the poult growth factor than that fed in the second and third experiments, although all samples of the product were obtained from the same manufacturer. Scott ('52) has reported earlier that distillers' dried solubles may vary in their content of the poult growth factor. This experiment was carried out in the summer months and the growth of the poults was not equivalent to that of the birds in the preceding test, described above.

Fifth experiment

The addition of DL-methionine (group 3), distillers' dried solubles (group 2) or bacitracin (group 4) to the basal diet produced a significant increase in the weights and a decided improvement in the feed efficiency of the poults in the 5th experiment (table 3). The addition of bacitracin and distillers' dried solubles (group 6), or a combination of this antibiotic and distillers' dried solubles with methionine, to the basal diet each produced a significant increase in growth and an improvement in feed efficiency. Bacitracin, distillers' dried solubles and methionine had an additive effect in increasing growth and improving feed efficiency.

Treatment of the data of this experiment by analysis of variance gave an F value of 22.95, whereas an F value of only 2.90 is necessary for significance at the 0.01 level of probability. The mortality observed in groups 1 and 3 was associated with the eating of litter by the poults during the first two weeks of the experimental period. As stated before, no explanation is available for the fact that the birds in these particular groups were more addicted to eating litter than those in other groups in the test.

DISCUSSION

From the results of this series of experiments it is quite apparent that DL-methionine produced an increase in growth

and an improvement in feed efficiency when added to an all-vegetable protein ration for turkey poults. Such a finding is in agreement with the studies of Clandinin et al. ('46) and Gerry, Carrick and Hauge ('48), where it was reported that chicks responded to additional methionine when fed an all-vegetable protein ration. It is thought that the growth response obtained by the addition of methionine to the ration in the present study was due to the presence of the free amino acid in the gastrointestinal tract, which can be readily utilized by the bird for necessary metabolic processes.

Distillers' dried solubles has been shown to contain an unidentified growth factor for the turkey poult, which is in agreement with the work of Novak, Hauge and Carrick ('47) and Scott ('52). Different samples of distillers' dried solubles obtained from the same manufacturer were found to vary in the poult growth factor content, which is in agreement with the work of Scott ('52).

Bacitracin and penicillin have been found to be of equal value for promoting the growth of turkey poults, either in the presence or in the absence of methionine and distillers' dried solubles. A lower level of penicillin than bacitracin is required for the maximum growth-promoting effect. An additive effect was obtained with the addition of the antibiotic, methionine and distillers' dried solubles to an all-vegetable protein ration for turkey poults.

Maximum growth was not obtained in experiments 1, 2, 4 and 5. It is believed that such decreased growth was due to the extremely hot weather which prevails in this area during the summer. However, it should be realized that these poults were hatched late in the season, and it is quite possible that they may not have had a carry-over of some nutritional factors, from the hen through the egg, which poults hatched earlier in the season might have had.

SUMMARY

From the results of this study it is apparent that bacitracin and penicillin will increase growth and improve feed efficiency

when added to an all-vegetable protein diet for turkey poults. It is indicated that antibiotics do not always induce a growth response under such conditions.

DL-methionine produced an increase in growth and an improvement in feed efficiency when 0.05% was added to the basal diet. This response was shown to be additive with that due to the unidentified factor content of distillers' dried solubles, and was further increased by the addition of an antibiotic to such a ration.

Distillers' dried solubles contain an unidentified poult growth factor. The content of such factor in this product varied in different samples obtained from the same manufacturer. No explanation of such variance is apparent from the data presented.

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The Broadbreasted Bronze turkey poults used in the 4th and 5th experiments were made available for this work as a grant-in-aid from the Janes Bar-Nothing Turkey Ranch.

The bacitracin used in the 4th and 5th experiments was supplied by S. B. Penick and Co., New York, New York.

The bacitracin used in the first, second and third experiments was supplied by the Commercial Solvents Corp., Terre Haute, Indiana.

The methionine used in all experiments was obtained through the courtesy of the Dow Chemical Co., Midland, Michigan.

The distillers' dried solubles and the procaine penicillin were supplied by Schenley Distillers, Inc., Cincinnati, Ohio.

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STUDIES ON OBESITY

II. FOOD INTAKE AND OXYGEN CONSUMPTION [1]

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The usefulness of highly inbred strains of mice in the study of obesity was demonstrated by Fenton and Dowling ('53). The feeding of certain purified diets, particularly those rich in fat, led to the development of obesity in C_3H strain mice by the time they were three months old. In mice of the C_{57} strain, on the other hand, excess fat was deposited much more slowly when a high fat diet was fed. It seemed to us that this strain difference in response to a high fat diet might be employed in the study of some factors contributing to obesity.

Several workers have reported greater weight gains or greater food consumption or both when experimental animals were fed diets of high fat content, as compared with the results when animals were fed low fat diets (Forbes et al., '46; Deuel et al., '47; Fenton and Carr, '51b; and others). Forbes et al. ('34) observed that in rats maintained at different levels of energy intake, the heat production increased as the food intake increased. The data presented in this paper show that both C_3H and C_{57} strain mice consume more calories when fed a 50% fat diet than when fed a 5% fat diet. Mice of the C_{57} strain responded to the increased caloric intake with increased oxygen consumption, and thus presum-

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ably increased heat production. On the other hand, C_3H strain mice showed no increase in oxygen uptake when fed the high fat diet, a factor likely to contribute to the obesity so readily induced in this strain.

EXPERIMENTAL METHOD

Male mice of the C_{57} and C_3H strains were weaned at 21 days of age. Throughout the experiment they were maintained in individual screen-bottomed cages in an air-conditioned animal room. The animals were fed either diet 284, containing 5% fat, or diet 273, containing 50% fat (table 1). Food and water were supplied ad libitum. Altogether 48 mice were employed in this experiment, 12 of each strain on each of the two diets.

TABLE 1

Composition of diets

COMPONENTS	DIET	
	284	273
	gm	*gm*
Casein	30	30
Dextrin	60	15
Salt mixture A [1]	5	5
Corn oil	5	5
Crisco	.	45

[1] Vitamin content and composition of salt mixture as described by Fenton and Carr ('51a).

These 4 groups of mice were used throughout the experiment. Food consumption was measured at weaning and again when the animals were 5 to 6 months old. Oxygen uptake was determined at age 55 to 85 days and again at 120 to 135 days. During the first three days following weaning no records of food consumption were kept, the animals thus being allowed a short period to adjust to the new caging and feeding conditions. This period of adjustment was followed by a 9-day study of food consumption with each of the 4 groups. When the animals were between 5 and 6 months old, another measurement of food intake was carried out. This time the

9-day period of food consumption study was preceded by a 7-day period of adjustment.

Oxygen consumption was measured by the method of Schwabe and Griffith ('38) at an environmental temperature of 27°C. All measurements of oxygen uptake were carried out between 8:00 A.M. and noon, the animal being permitted one hour for adjustment to the apparatus. Oxygen uptake was then determined for a period of 30 minutes. The kymograph tracing was used to calculate the total oxygen uptake for the 30-minute run. It also permitted the calculation of oxygen consumption during periods of low activity. Measurements were carried out when the animals were between 55 and 85 days of age, and again between 120 and 135 days.

RESULTS

The data on food intake and weight gain are shown in table 2. On both the low and the high fat diet mice of the C_3H strain consumed more food per day than did the C_{57} strain animals. Consequently, mice of the C_3H strain gained weight at a faster rate than did those of the C_{57} strain. No

TABLE 2

Food intake of two strains of mice on low and high fat diets

STRAIN	DIET	FAT LEVEL	INITIAL WEIGHT	WEIGHT GAIN/9 DAYS	FOOD CONSUMPTION		WEIGHT GAIN/GM FOOD	WEIGHT GAIN/ 100 CAL.
		%	gm	gm	gm	Cal.	gm	gm
				Mice aged 21 days				
C_{57}	284	5	10.3	4.6 ± 0.20[1]	24.9	100.8 ± 3.7	0.185	4.57 ± 0.30
C_{57}	273	50	10.5	6.1 ± 0.28	18.0	113.6 ± 3.7	0.340	5.41 ± 0.63
C_3H	284	5	10.8	4.9 ± 0.39	28.6	115.8 ± 2.2	0.172	4.24 ± 0.29
C_3H	273	50	10.9	7.3 ± 0.27	21.4	134.3 ± 5.7	0.342	5.45 ± 0.30
				Mice aged 5–6 months				
C_{57}	284	5	25.6	0 ± 0.27	26.1	104.3 ± 2.8	....	.....
C_{57}	273	50	29.4	1.4 ± 0.17	19.2	121.0 ± 3.7	....	.....
C_3H	284	5	30.4	0.6 ± 0.37	32.5	130.0 ± 4.3	....	.....
C_3H	273	50	36.8	2.0 ± 0.19	23.4	147.2 ± 4.8	....	.....

[1] Standard error.

strain difference was apparent with respect to efficiency of food utilization for growth.

Both strains of mice consumed more calories when fed the 50% fat diet than they did when fed the low fat ration. Each strain showed a higher efficiency of food utilization on the high fat diet. Efficiency of food utilization was calculated only for the weanling mice, since their starting weights were nearly identical. The 4 groups of animals showed widely different weights at 5 to 6 months of age, and therefore had different maintenance requirements. Thus, efficiency of utilization of food for weight gain could not be satisfactorily calculated.

TABLE 3

Oxygen uptake of two strains of mice on low and high fat diets

STRAIN	DIET	FAT LEVEL	OXYGEN UPTAKE — ML/HOUR/GM	
		%	"Inactive"	"Average"
		Mice aged 55–85 days		
C_{57}	284	5	2.84 ± 0.10 [1]	3.24 ± 0.12
C_{57}	273	50	3.38 ± 0.14	3.78 ± 0.14
C_3H	284	5	3.01 ± 0.10	3.33 ± 0.13
C_3H	273	50	2.89 ± 0.09	3.15 ± 0.09
		Mice aged 120–135 days		
C_{57}	284	5	3.09 ± 0.09	3.46 ± 0.15
C_{57}	273	50	3.48 ± 0.11	4.09 ± 0.19
C_3H	284	5	2.94 ± 0.11	3.25 ± 0.16
C_3H	273	50	2.69 ± 0.13	3.05 ± 0.12

[1] Standard error.

Table 3 shows the oxygen uptake of C_{57} and C_3H strain mice on the low and the high fat diets. At the 5% fat level there was no significant strain difference with respect to oxygen uptake. Mice of the C_{57} strain fed the 50% fat diet consumed significantly more oxygen than did the animals on the low fat intake. In contrast, the C_3H strain mice fed the high fat rations consumed no more oxygen than did mice of the same strain fed the low fat diet. In fact, the high fat ration seemed to reduce the oxygen uptake of the C_3H strain mice. The decrease, however, was not statistically significant.

DISCUSSION

Mice of the C_{57} and C_3H strains voluntarily consumed more calories when fed a 50% fat ration than when fed a 5% fat diet. This is in agreement with the findings of Deuel et al. ('47) and others. From the work of Forbes et al. ('34) it might be expected that this increased caloric intake would result in increased oxygen consumption. This fact proved to be true for mice of the C_{57} strain. In contrast, however, were the findings with animals of the C_3H strain; these consumed no more oxygen when fed the high fat ration, although their caloric intake was significantly greater than that for the C_3H strain mice on the low fat diet. Thus it appears that mice of the C_{57} strain possess a mechanism for the oxidation of the extra calories consumed on the high fat diet. This mechanism seems to be lacking, or at least poorly developed, in mice of the C_3H strain. The latter thus seem to be forced to deposit the extra calories consumed as carcass fat. That this is indeed the case has been demonstrated in an earlier paper by Fenton and Dowling ('53).

CONCLUSIONS

1. Mice of the C_{57} and C_3H strains consumed more calories when fed a 50% fat diet than when fed a 5% fat ration.
2. Mice of the C_{57} strain consumed more oxygen at the 50% fat level than at the 5% fat level. Thus the extra caloric intake on the high fat diet was oxidized, at least in part.
3. In C_3H strain mice oxygen uptake was not increased in response to the greater caloric intake on the high fat diet. The extra calories consumed were deposited as carcass fat.

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RELATION OF DIETARY PROTEIN LEVELS TO REPRODUCTION IN THE RAT [1]

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This study on the relation of dietary protein levels to reproduction in female rats has been undertaken because (1) no study on this problem has been carried out using purified diets supplemented with all dietary essentials known at the present time and (2) no measurement of food intake on a protein-free diet is available, so that the degree of undernutrition under such conditions is not known. The careful work with adult rats by Guilbert and Goss ('32) on low protein levels during reproduction and that by Seegers ('37) on protein-free diets during gestation were carried out before modern knowledge on the vitamin B complex was available.

EXPERIMENTAL

Normal female rats (Long-Evans strain), 80 to 100 days of age and approximately 200 gm in body weight, were bred with normal males and placed on the purified diets varying in protein level the day of mating, i.e., the day of finding sperm. Later, additional groups of animals were placed on the protein-free diet before mating or during gestation. Vaginal smears

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were examined daily during gestation for the presence of erythrocytes, the sign of implantation; all rats were weighed at regular intervals. The animals were kept on screens until the day before parturition was expected to occur. All rats were autopsied a few hours after parturition had taken place, unless weight loss or other signs indicated that resorption had occurred; in such cases the autopsies were usually performed on day 21 of gestation. At autopsy the uterus was carefully examined for the presence of implantation sites to determine whether the sites were normal or in the process of resorption.

The basal purified diet containing 20% protein is the standard control diet used in this laboratory. It consists of 24% alcohol-extracted casein, 64% sucrose, 8% hydrogenated cottonseed oil,[2] and 4% salts.[3] Crystalline vitamins per kilogram of diet are: 300 μg *d*-biotin, 5 mg 2-methyl-1, 4-naphthoquinone, 5 mg thiamine HCl, 5 mg pyridoxine HCl, 5.5 mg pteroylglutamic acid, 10 mg riboflavin, 10 mg *p*-aminobenzoic acid, 20 mg niacin, 50 mg *d*-calcium pantothenate, 400 mg inositol, and 1.0 gm choline chloride. All rats received weekly a fat-soluble vitamin mixture containing 800 U.S.P. units vitamin A, 115 chick units vitamin D,[4] 6 mg synthetic dl-alpha-tocopherol, and 650 mg corn oil.[5] The alcohol-extracted casein used in this

[2] Crisco or Primex.

[3] Salts 4 of Hegsted et al. ('41).

[4] Furnished by high-potency Sardilene (Booth Fisheries, Seattle, Washington) containing 15,000 I.U. vitamin A and 2,500 units vitamin D per gram.

[5] Mazola.

[6] The alcohol-extracted casein used in this study was prepared from commercial technical grade casein by extraction with hot 95% alcohol for 4 hours, with cold 70% alcohol twice for 24 hours, and with cold 95% alcohol for 24 hours. The nitrogen content of this casein sampled over a period of two years, as determined by the micro-Kjeldahl method, averaged 13.3 to 13.4%. For comparison, samples of GBI or of Labco casein analyzed at the same time averaged 14.1% nitrogen. Multiplying the nitrogen content by the factor 6.25 (instead of 6.38, the factor usually used for milk proteins) expresses the results in terms of protein containing 16% nitrogen and facilitates comparison between different casein preparations and preparations of other proteins. Thus, the alcohol-extracted casein averaged 83.4% protein in comparison with GBI and Labco caseins, which averaged 88.1% protein.

study contained 83% crude protein (N × 6.25).[6] Casein and sucrose were interchanged as the protein level varied from 25% to 0%. Casein levels of 0, 3, 6, 12, 18, 24, and 30% were used, corresponding to protein levels of 0, 2.5, 5, 10, 15, 20, and 25%, respectively. The amount of crystalline vitamins was doubled and 50 μg vitamin B_{12} per kilogram incorporated in the diet for half the animals on the diets containing low protein levels (5% and 2.5%) and for all animals on the protein-free diet. (Our stock diet apparently contains sufficient B_{12} for short-term experiments with stock animals at the usual casein levels.)

RESULTS

Table 1 summarizes the reproductive performances of rats placed on the purified diets, varying in protein level from 25% to 0%, on the day of mating. As the protein level was dropped from 25 to 20 to 15 and finally to 10%, few adverse effects on reproduction could be noted. Vaginal RBC, the appearance of erythrocytes in the vaginal smear, occurred normally 12 to 13 days after breeding, with few exceptions. All rats showed 100% implantations and litters with a normal number of young per litter (8 to 10) and a normal birth weight, 6 gm. The tendency toward a decreased average maternal weight gain during gestation with decreasing protein level may be noted, i.e., from 120 gm to 82 gm, and a drop of the birth weight of the young to less than 6 gm at the 10% protein level.

Decreasing the protein level to 5% (6% casein) resulted in 30% resorptions and marked decreases in average maternal weight gain, i.e., from 82 gm to 11 gm during the 20-day gestation period. The young produced by mothers on this diet weighed less than 5 gm and 17% were stillborn. When the crystalline vitamins were doubled and B_{12} included in the diet (starred group), reproductive performance improved, as shown by the lack of resorptions and the decreased incidence of stillborn young, only 2%. On the 2.5% protein level with the basal vitamin supplementation, 90% of the mothers re-

TABLE 1

Relation of dietary protein levels to reproduction in the rat

DIETARY PROTEIN LEVEL	RATS BRED	WT. CHANGE DURING GESTATION [1]	VAGINAL RBC [2]	IMPLANTATIONS	RESORPTIONS OF IMPLANTATIONS	LITTERS OF IMPLANTATIONS	YOUNG	
							Ave. wt.	Born dead
%	no.	gm	day	%	%	%	gm	%
25	10	+ 120	12.4	100	0	100	6.5	1
20	10	+ 116	11.7	100	0	100	6.0	6
15	10	+ 95	12.6	100	0	100	6.0	1
10	10	+ 82	12.6	100	0	100	5.5	1
5	10	+ 11	11.5	100	30	70	4.7	17
	10 [3]	+ 24	12.6	100	0	100	4.7	2
2.5	10	— 32	10.2	100	90	10	3.4	0
	10 [3]	— 22	11.0	100	70	30	3.8	0
0	10 [3]	— 41	10.6	90	89	11	2.9	0
	10 [3]	— 41	9.8	100	100	0		

[1] Data determined during the 20 days following mating.

[2] Day when vaginal smear first contained erythrocytes (RBC).

[3] The amount of crystalline vitamins was doubled and vitamin B_{12} added to the diet for these groups.

sorbed, vaginal RBC occurred on day 10, and the mothers lost an average of 32 gm during gestation. A further decrease in the birth weight of young to 3.4 gm may also be noted. With doubled vitamins and the inclusion of B_{12}, reproductive performance was improved on this very low protein level, as shown by the decreased incidence of resorptions, improvement in the birth weight of young to 3.8 gm, and an appearance of vaginal RBC on day 11. When the protein-free diet was employed, the animals consistently showed 90 to 100% resorptions, with vaginal RBC on day 10. An average loss in maternal body weight of 41 gm was observed. The few young produced on this diet were small in size and weighed less than 3 gm.

An extremely rapid resorption of implantation sites was noted for animals on the protein-free diet, the 2.5% protein diet, and sometimes for the 5% protein diet. This process was carried out so rapidly that when animals were autopsied at the end of gestation, days 21 to 23, no trace of implantation sites could be observed in the majority of rats. Doubling the crystalline vitamins and including B_{12} in the 5 and 2.5% protein diets apparently resulted in slightly later fetal death and prolongation of placental life, so that more implantation sites could be observed at autopsy in the starred groups. However, the average number of implantation sites macroscopically detectable for the protein-free and the 2.5% protein diets with increased vitamin supplementation was only 5.3, in comparison with the normal values of 9 to 10 observed in all groups above the 5% protein level.

Comparison of food restriction and the absence of protein

To eliminate the factor of undernutrition or restriction of food intake as a cause of the reproductive failure on the protein-free diet, paired-feeding studies were carried out. Normal female rats, 80 to 85 days of age and averaging 195 gm in body weight, were bred with normal males and divided into three equivalent groups, the rats being carefully

paired with regard to body weight on the day of breeding. One group received the protein-free diet, with food intake being measured daily to 0.1 gm; one group received the 20% protein diet with doubled vitamins and B_{12} in the same amount as was consumed by the corresponding rats on the protein-free diet the same day of gestation; the last group received ad libitum the 20% protein diet containing the basal vitamin supplementation.

Table 2 summarizes the experimental data. On the protein-free diet 90% of the animals implanted and resorbed; with an average daily food intake of 9.2 gm, or 56% of the ad libitum intake, the mothers lost 41 gm during gestation and vaginal RBC appeared betweens days 10 and 11. The pair-fed controls showed 90% implantations and litters, with 10% resorptions. These mothers gained 32 gm in body weight, vaginal RBC appeared later between days 11 and 12, and the young were heavier at birth, although not up to the normal birth weight. Ad libitum controls were normal in reproductive performance. Since the pair-fed controls were slightly below normal, especially in regard to the occurrence of resorption, the protein level was increased to 30% (36% casein). With this increase in protein level, reproduction in the pair-fed control group was normal, i.e., 100% litters, normal appearance of vaginal RBC between days 12 and 13, and normal birth weight of the young, 5.7 gm.

Effect of instituting the protein-free diet before and during gestation

Macroscopic observations of implantation sites during pregnancy on the protein-free diet revealed that fetal death had occurred on or before day 10 in the majority of rats. Since this is less than 4 days after implantation, short periods of deficiency prior to breeding were used in an attempt to interfere with implantation. It was found that the majority of rats had only one estrous cycle after institution of a protein-free diet and would frequently not mate, even when in apparent estrus as judged by vaginal smears. Three to 7 days

TABLE 2

Relation of food restriction to reproduction on a protein-free diet

EXPTL. GROUP	RATS BRED	AVE. DAILY FOOD INTAKE	WT. CHANGE DURING GESTATION	VAGINAL EBC	IMPLANTATIONS	RESORPTIONS OF IMPLANTATIONS	LITTERS OF IMPLANTATIONS	YOUNG AVE. WT.
	no.	*gm*	*gm*	*day*	%	%	%	*gm*
0% Protein	10	9.2	— 41	10.6	90	89	11	2.9
20% Protein, pair-fed	10	9.2	+ 32	11.8	90	11	89	4.8
20% Protein, ad libitum	10	16.5	+ 108	12.4	100	0	100	5.8
30% Protein, pair-fed	10	9.2	+ 42	12.7	100	0	100	5.7
30% Protein, ad libitum	10	16.4	+ 117	12.7	100	0	100	6.1

of deficiency prior to mating resulted in only a slight further impairment of reproduction, as shown by 80% implantations and 100% resorptions (table 3). However, institution of the same protein-free diet after implantation had occurred, namely, 7 days after mating, resulted in normal implantation and 90% litters, thus indicating the critical need for protein during the early stages of placental and embryonic development.

To confirm these findings, a study of the effects of transitory protein deficiency was carried out. The animals were placed on the protein-free diet for the designated days and then shifted to the control diet containing 20% protein for the remainder of the pregnancy. All dietary shifts were made at 10:00 A.M. Table 4 shows that maintenance on the protein-free diet for the first 7 or 8 days of pregnancy resulted in 20 to 25% resorptions. Extending the deficiency period for only one or two days, namely for the first 9 or 10 days, resulted in 86 to 100% resorptions, with early appearance of vaginal RBC. These findings confirm and extend the conclusions from macroscopic studies of implantation sites that fetal death on the protein-free diet occurred on or before day 9 or 10 in the majority of rats. The greater need for dietary protein during the early stages of pregnancy is further shown by instituting the 9-day period of protein-free diet somewhat later in gestation. When the 9-day period started the day of mating (group 0–9), 86% resorptions resulted; when the 9-day period started three days after mating (group 3–12) 50% resorptions resulted; when it was delayed to 5 days after mating (group 5–14), only 20% resorptions were observed.

Impaired implantation was noted in some of the groups with transitory protein deficiency and occasionally in other groups on the protein-free diet. It should be mentioned that the rapidity of the resorptive process in animals on the protein-free diet renders difficult the macroscopic observation of implantation sites as evidence of implantation. In the groups with transitory protein deficiency, this rapid process is accelerated by the shift to the control diet, with consequent

TABLE 3

Effect of protein-free diet before and during gestation

PROTEIN-FREE DIET STARTED	RATS BRED	WT. CHANGE DURING GESTATION	VAGINAL EBC	IMPLANTATIONS	RESORPTIONS OF IMPLANTATIONS	LITTERS OF IMPLANTATIONS	YOUNG	
							Ave. wt.	Born dead
	no.	*gm*	*day*	*%*	*%*	*%*	*gm*	*%*
3–7 Days before breeding	10	—27	9.9	80	100	0		
Day of breeding	20	—41	10.2	95	95	5	2.9	0
7 Days after breeding	12	+ 1	12.8	100	8	92	3.8	17

TABLE 4

Effect of transitory protein deficiency during gestation

PROTEIN-FREE PERIOD	RATS BRED	WT. CHANGE DURING GESTATION	VAGINAL EBC	IMPLANTATIONS	RESORPTIONS OF IMPLANTATIONS	LITTERS OF IMPLANTATIONS
days	*no.*	*gm*	*day*	*%*	*%*	*%*
0– 7	5	+ 66	12.4	100	20	80
0– 8	5	+ 70	12.3	80	25	75
0– 9	10	+ 41	10.4	70	86	14
0–10	10	+ 23	10.4	80	100	0
3–12	10	+ 43	11.6	100	50	50
5–14	10	+ 67	12.4	100	20	80

restoration of the normal estrous cycle after fetal and placental death. Consequently, the majority of rats receiving the protein-free diet the first 9 to 10 days did not exhibit any observable implantation sites at autopsy, and the only evidence for implantation in such rats was the appearance of erythrocytes in the vaginal smear.

No young with macroscopic congenital abnormalities were observed in any group receiving the low protein or protein-free diets.

DISCUSSION

The data presented show that the 5% protein level is apparently the critical level under our experimental conditions. Above this level reproductive performance is normal and below this level reproduction is severely disturbed. This finding is in agreement with the work of Guilbert and Goss ('32), who used somewhat different experimental conditions, i.e., a modified stock diet, varying sources of protein, rats from 50 to 100 days of age, and maintenance on the low-protein diets before mating — but who likewise found that approximately 5% protein was the critical level. In long-term experiments in which weanling rats were used Macomber ('33) similarly found that reduction of the protein level to 5% did not materially affect pregnancy, although "fertility" was reduced. This low minimum requirement for protein for reproduction is in striking contrast to the dominant role of protein in lactation for the rat, e.g., Macomber ('33); Mueller and Cox ('46).

Pregnancy on the protein-free and on the 2.5% protein diets was characterized by a high incidence of resorptions (70 to 100%), early fetal death as indicated by the early appearance of vaginal erythrocytes, an exceedingly rapid resorptive process so that frequently no implantation sites could be observed at autopsy, and marked decreases in maternal body weight. The occurrence of resorptions together with early fetal death and early appearance of vaginal erythrocytes had been observed previously on low protein diets by Guilbert and Goss ('32). The general picture, with the exception of

the marked loss in maternal weight, is similar to that observed during pregnancy in the B_6-deficient rat (Nelson and Evans, '48, '51) and in the hypophysectomized rat (Pencharz and Long, '33), and suggests the possibility of hormonal deficiencies in these animals.

The necessity of increasing the protein level of the pair-fed control diet to 30% for normal reproductive performance was unexpected, although it is customary in studies on vitamin deficiencies to double or triple the intake of all vitamins for pair-fed controls. Guilbert and Goss ('32) concluded that quantitative undernutrition was not a factor in reproductive disturbances on their low protein diets, as the food intake of such rats varied from a minimum of 9.2 gm for one rat daily to the commonly observed average of 12.5 to 14.8 gm daily. The restriction in food intake observed on the protein-free diet in the present study was much more severe, the intake varying from 7.8 to 11.0 gm daily per rat and averaging 9.2 gm, or 56% of the ad libitum intake. This degree of dietary restriction is also more severe than that observed previously for pterolyglutamic acid deficiency during pregnancy, 79% of the ad libitum intake (Nelson and Evans, '49), or for pantothenic acid-deficient gestations, 69% of ad libitum intake (Nelson and Evans, '46), but is approximately the same as that observed for B_6-deficient gestations, i.e., 9.5 gm or 56% of the ad libitum intake (Nelson and Evans, '51). In the study on B_6 deficiency, rats averaging 110 days of age were used, whereas younger rats averaging 80 days of age have been used in this study on protein-free diets. The younger rats would presumably have a higher protein requirement for growth superimposed upon the requirement for pregnancy. However, these younger rats showed normal reproductive performance (100% litters) at the 5 and 10% protein levels, on which their average daily protein intake must have been less than that of the 20% protein pair-fed controls, where only 90% litters were observed. The daily food and caloric intakes on the 5 and 10% protein diets would, of course, be greater than those of the 20% protein pair-fed controls.

An analysis of the average food intake for 5-day periods during gestation showed that the pattern of dietary restriction for protein deficiency differed from that observed for B_6 deficiency (and for the corresponding pair-fed controls):

	Protein-free diet gm	*B_6-deficient diet* gm
Days 1–5	8.8	11.0
Days 6–10	8.7	8.8
Days 11–15	9.8	9.4
Days 16–20	9.6	9.6

It may be noted that the food intake for the first 5 days of gestation (and also for the first 10 days) was much lower on the protein-free diet than on the B_6-deficient diet. During the remainder of gestation the food intakes on both deficiencies were similar; also in both deficiencies an increased food intake occurred after the second quarter of pregnancy, days 6 to 10. During these 5 critical days (days 6 to 10) implantation occurs and the early stages of placental and fetal development are being carried out in the normal rat, whereas in both deficiencies fetal death has occurred in the majority of rats by the end of the period, day 10. Thus, not only the average reduction in food intake but also the severity of food restriction during the first half of pregnancy are important factors in determining the outcome of gestation.

The critical need for protein during the early stages of placental and fetal development was also emphasized by the results obtained when the timing or duration of the protein-free diet was varied. In contrast to the consistent reproductive failure (90 to 100% resorptions) observed when the protein-free diet was started the day of mating, 90% litters resulted when the diet was delayed until after implantation, only 7 days later. Such results differ from those reported by Seegers ('37), who found that rats placed on a protein-free diet before the 9th day rarely produced litters, while those placed on the same diet the 12th day usually did. The difference in reproductive success can probably be explained by the greater protein stores of our stock rats before mating, and possibly

also by better vitamin supplementation of the protein-free diet used in this study.

It may be mentioned that the rapidity with which the low protein and protein-free diets disturb reproductive performance is greater than that found for any vitamin deficiency studied so far (without a specific antagonist), even that of thiamine (Nelson and Evans, unpublished data).

SUMMARY

The relation of dietary protein levels to reproduction has been studied in adult rats placed the day of breeding on purified diets supplemented with all known dietary essentials and containing casein as the source of protein. Under these conditions the 5% protein (6% casein) level was apparently the critical level, as above this level reproductive performance was normal but below it reproduction was severely disturbed.

Pregnancy on the protein-free and low protein (2.5%) diets was characterized by a high incidence (70 to 100%) and early occurrence of resorptions. The resorptive process was rapid and implantation sites were often not detectable at the end of gestation. Marked decreases in maternal body weight also occurred. With the exception of loss of maternal body weight, the general picture is similar to that observed for pregnancy in the B_6-deficient rat and in the hypophysectomized rat, and suggests the possibility of hormonal deficiencies in these animals.

The critical need for protein during the earliest stages of placental and fetal development is in contrast with the reproductive success which resulted when a protein deficiency was instituted only shortly after implantation.

The normality of pair-fed controls when the protein level was raised to 30% eliminated the factors of food restriction and of other dietary deficiencies as causes for these reproductive disturbances.

No young with macroscopic congenital abnormalities were observed in any group on the protein-free diet.

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DISTRIBUTION OF THE XANTHINE OXIDASE FACTOR (MOLYBDENUM) IN FOODS[1]

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ONE FIGURE

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Previous studies from this laboratory (Westerfeld and Richert, '51, '52; Richert and Westerfeld, '51) have demonstrated the dependence of intestinal xanthine oxidase (XO) on an unidentified dietary constituent which was referred to as the "liver residue" or "xanthine oxidase factor" (XOF). This factor has recently been identified as the trace element molybdenum (Westerfeld and Richert, '53; Richert and Westerfeld, '53; DeRenzo et al., '53). The present report describes the distribution of XOF in a large number of foods, as measured by the bioassay procedure (Richert and Westerfeld, '51). Many foods were also analyzed for molybdenum chemically, and a comparison of the two values showed that while the assay results generally paralleled the total molybdenum content, only a portion of the total molybdenum present in foods was usually available for this biological response.

EXPERIMENTAL

For the bioassay procedure, weanling male rats were obtained[2] and placed on a purified 24% casein diet[3] for 6 to

[1] This study was aided by grants from the Nutrition Foundation, Inc., and the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[2] From Albino Farms.

[3] Labco. Labco casein was found (Westerfeld and Richert, '53) to be superior to the GBI casein previously used.

10 days to deplete the intestine of xanthine oxidase (Westerfeld and Richert, '51). Six rats were then fed the test diet for 7 days before they were sacrificed; the intestines were analyzed for xanthine oxidase by the manometric procedure previously described (Westerfeld and Richert, '51). The average intestinal xanthine oxidase value found for each test diet was used to estimate the "available" molybdenum in the food by reference to the assay curve obtained with known amounts of sodium molybdate. The average intestinal XO response of 30 groups on the control diet alone was 1.65, with a maximum upper limit of 3 (mm^3 O_2 per 20 minutes per flask containing 283 mg fresh tissue).

Edible portions of foodstuffs were assayed at 10% of the diet by substituting them for an equal amount of glucose. Fresh foods were either dried extensively at 65° before addition to the diet or the water content of the food was allowed for, so that all additions were made on a dry weight basis. All diets contained a minimum of 24% casein; a test supplement consisting of 70% protein would increase this value to 31% protein. Such variations were without effect, since the same assay curve was obtained when the diet contained 24% or 31% casein.

Foods were analyzed for molybdenum by digesting 3 to 30 gm of the dry sample with concentrated H_2SO_4, redistilled HNO_3 and perchloric acid (Bertrand, '43). The clear digest was diluted with water, neutralized to phenolphthalein with an excess of concentrated NH_4OH and evaporated to a small volume to remove excess ammonia. The solution was then diluted to 50 ml, 7 ml of concentrated HCl were added, and the molybdenum color was developed with KCNS and $SnCl_2$ as described by Marmoy ('39). After 5 minutes (Teresi et al., '42), the color was extracted with ether and read on a Coleman spectrophotometer at 470 mμ for comparison with a standard curve. From 92 to 100% of an added 6 μg Mo were recovered by these procedures.

RESULTS

Figure 1 shows a comparison between the intestinal XO response to sodium molybdate and liver residue. The very high values (averaging up to 45) obtained with the former have seldom been obtained with natural foodstuffs which contained much more than the same amount of molybdenum.

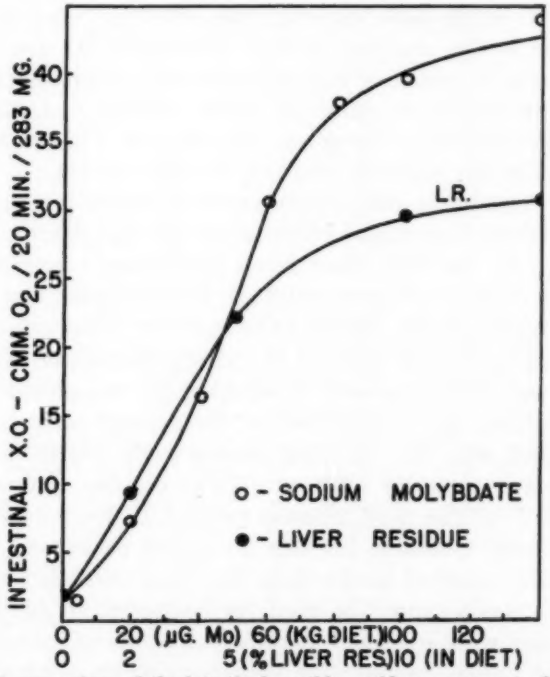

Fig. 1 A comparison of the intestinal xanthine oxidase response to dietary sodium molybdate and liver residue.

Even with sodium molybdate, assay responses above 30 were somewhat erratic and have therefore been considered to be "saturation" levels of intestinal XO. Assays falling within this range were usually repeated at lower dietary concentrations. Since the liver residue contained 1.82 μg of molybdenum per gram by chemical analysis, it is evident that all of the

molybdenum present in this material was not available for the biological response even at the lower levels of intestinal XO. Whether the molybdenum available for this response is synonymous with "absorbable" molybdenum is as yet unknown. It seems improbable that molybdenum which is unavailable for absorption into the intestinal mucosa could be absorbed through it, but it is possible that more dietary molybdenum could pass through the intestine than would be indicated by the absolute level of intestinal XO observed. It is tempting to consider the molybdenum-xanthine oxidase relationship in the intestine as being analogous to the iron-ferritin relationship (Granick, '51), and no evidence has been obtained so far which is contrary to this concept.

Table 1 shows a comparison between the molybdenum content of foods determined chemically and that determined biologically. In general, those foods containing relatively large amounts of Mo were most active in the biological test, but the Mo in foods usually did not have as much effect on intestinal XO as an equivalent amount of sodium molybdate. When the percentage of "biologically available" Mo was plotted against the intestinal xanthine oxidase or the amount of liver residue in the diet, and the resulting curves were extrapolated to a zero enzyme activity or a zero liver residue concentration, 85 to 90% of the molybdenum present in liver residue was theoretically available for this biological response. The single values reported in the table for the availability of Mo in different foods cannot be used for comparative purposes because they were obtained at different levels of intestinal XO response. Such comparative values might be obtained by assaying each food at several different levels and extrapolating the response curve to a zero enzyme activity. There were two foods, kidney beans and lentils, in which the Mo content was relatively high but unavailable biologically in repeated assays; these results are considered significant because only a small percentage of the dietary Mo was reflected in the assay, even when the intestinal XO did not approach saturation levels.

TABLE 1

A comparison between the Mo content of foods determined chemically and the biological response as determined by the level of intestinal xanthine oxidase

SUBSTANCE TESTED	Mo CONTENT, μG/GM	ASSAY CONDITIONS		ASSAY RESULTS		% OF DIETARY Mo ACTIVE IN TESTS
		% of test subst. in diet	Mo in diet, μg/kg	Intestinal X. O.	Mo in diet μg/kg	
Liver residue	1.82	2	36	10	26	72
		5	91	22	48	53
		10	182	29	58	32
		15	273	31	61	22
Soy flour (extracted)	2.59	3	78	30	60	77
		10	455	33	65	..
Pea beans	4.55	2	91	40	100	100
		10	469	27	55	..
Baby lima beans	4.69	2	94	35	71	76
		10	138	31	61	44
Split green peas	1.38	5	69	32	63	91
		10	111	33	65	59
Split yellow peas	1.11	6	67	22	48	72
Lentils	1.20	10	120	18	42	35
Red kidney beans	0.91	10	91	5	14	15
		10	717	42	150	..
Brewers' grain concentrate	7.17	1	72	30	59	82
Wheat germ (defat.)	1.34	5	67	28	57	85
Whole wheat flour	0.64	10	64	23	50	78
Patent wheat flour	0.37	10	37	15	38	100
Puffed wheat	0.70	10	70	14	36	51
Macaroni	0.51	10	51	18	43	84
		10	107	30	59	55
All bran (wheat)	1.07	6	64	25	52	81
Buckwheat flour	0.08	10	8	5	14	..
White rice	1.14	10	114	38	84	81
		10	103	28	57	55
Brown rice	1.03	6	62	32	63	100
Puffed rice	0.38	10	38	8	22	58
Barley	0.52	10	52	11	30	58
		10	162	28	57	35
Dark rye flour	1.62	4	65	13	34	52
Mothers oats	1.14	6	68	29	58	85
Yellow corn meal (degerm.)	0.06	10	6	4	11	..
White corn meal (degerm.)	0.12	10	12	3	8	..
Corn flakes	0.08	10	8	1	0	..
Hog kidney (defat.)	3.47	2	69	28	56	81
Calf spleen (defat.)	0.93	7	65	29	58	89
Duodenum (defat.)	0.32	10	32	5	15	47
Horse heart (defat.)	0.05	10	5	3	7	..
Dry beef	0.07	10	7	3	8	..
Dry whole milk (Klim)	0.26	10	26	12	32	100
Dry skim milk (Starlac)	0.27	10	27	5	14	52
Whey	0.34	10	34	12	32	94
Egg yolk	0.17	10	17	3	8	..
Egg albumin	0.07	10	7	3	8	..
Cerophyl	3.37	3	101	29	58	57
Alfalfa	0.35	10	35	9	23	66
Red cabbage	1.27	10	127	36	75	59
Hubbard squash	1.05	10	105	24	51	49
Onions (no. 1)	0.54	10	54	22	48	89
Onions (no. 2)	0.14	10	14	2	5	..
Cantaloupe	0.16	10	16	6	16	100
Tapioca	0.03	10	3	1	0	..
Raisins	0.13	10	13	1	0	..
Apricots	0.14	10	14	2	4	..
Cocoanut	0.29	10	29	10	28	97
Peanuts	0.25	10	25	5	14	56
Cocoa	0.17	10	17	1	0	..
Brewers' yeast	1.33	5	67	22	49	73
Brewers' yeast	0.85	10	85	25	52	61
Bakers' yeast (Budweiser)	0.08	10	8	2	4	..
Bakers' yeast (Fleischman)	0.09	10	9	2	4	..
Bakers' yeast (National)	0.05	10	5	2	4	..
Difco yeast extract	5.57	10	557	31	61	..
		1	56	25	52	93

Table 2 shows the bioassay values for additional foods which were not analyzed chemically. Foods containing less than 8 μg of available Mo per 100 gm solids could be considered negative in the bioassay when tested at 10% of the diet, since the intestinal XO response at this Mo concentration was at the upper level of variation obtained with the control diet alone. The recorded values are representative of the "available" Mo in these foods, rather than of total molybdenum. Foods considered to be good or excellent sources of XOF contained 0.6 or more p.p.m. of available Mo on a dry weight basis. Inactive or trace foods contained less than 0.1 p.p.m. For comparison, purified milk xanthine oxidase had a Mo content of 300 p.p.m. and this represented a 1,000-fold concentration of the Mo in the original milk.

In general, foods containing relatively large amounts of Mo were (1) most cereals, grains, (2) legumes, and (3) some dark green leafy vegetables. Animal tissues highest in Mo included liver, kidney and spleen; muscle meats and fish contained little Mo. Fruits, berries and most root or stem vegetables were relatively poor sources of Mo.

Thirty-two dried yeast samples [4] were also assayed at 10% of the diet, and several were analyzed chemically (table 1). The XOF content of the yeast varied with the type of yeast and the growth medium. Strain T yeasts (torula) had good activity (42 to 47 μg Mo/100 gm) when grown on molasses with or without corn steep solids, but were inactive (0 to 4 μg Mo) when grown on sulfite waste liquor. Strain H yeasts (cerevisiae) were inactive (0 to 11 μg Mo) when grown initially on the molasses-corn steep medium, but appeared to contain small amounts of this factor (17 to 36 μg Mo) when transferred serially through media containing corn steep solids. Those yeasts which were active obviously abstracted molybdenum from the growth medium, even though molasses and corn contained only small quantities of XOF. Cerevisiae baking yeasts grown on molasses plus corn steep solids were

[4] We are indebted to Dr. Henry Buehler of Anheuser-Busch, Inc., for supplying the yeasts.

TABLE 2

The "available" molybdenum in foods as determined by the intestinal bioassay procedure for the xanthine oxidase factor

(Tested at 10% of diet on a dry weight basis)

SUBSTANCE TESTED	μG Mo/100 GM DRY SOLIDS	SUBSTANCE TESTED	μG Mo/100 GM DRY SOLIDS
Vegetables		*Fish*	
Lettuce	0	Salmon	0
Watercress	0	Halibut	0
Beets	4	Mackerel	0
Beet greens	50	Lobster	0
Carrots	22	Shrimp	5
Carrot greens	27	Oysters	9
Parsnips	0	Tuna fish	4
Parsnip greens	14	Haddock	8
Cabbage	56	Clams	14
Chinese cabbage	32	Scallops	36
Cauliflower	27	Scallops	52
Brussels sprouts	11	*Meats*	
Broccoli	53	Veal chop	0
Spinach	20	Shoulder lamb	0
Asparagus	0	Ham	0
Celery	12	Veal cutlet	0
Endive	22	Turkey legs	5
Rutabaga	11	Turkey breast	8
Turnips	22	Tenderloin steak	6
Egg plant	0	Lamb chops	4
Green peppers	27	Pork loin	8
Pumpkin	20	Hamburger	4
Potatoes	48	Chicken legs	17
Sweet potato	0	Chicken breast	17
Tomatoes	6	Chicken heart	28
Mushrooms	27	Chicken liver	51
Corn	8	Beef kidney	44
Green beans	56	Pork liver	51
Fruits and berries		*Milk products*	
Cranberries	0	Cottage cheese	8
Figs	0	Cream cheese	11
Peaches	0	Swiss cheese	11
Pineapple	0	Butter	8
Apples	8	*Nuts*	
Pears	0	Pecans	0
Bananas	0	Cashew nuts	14
Cherries (sweet)	4	Almonds	25
Prunes	0	Walnuts	0
Watermelon	0	Brazil nuts	2
Blueberries	0	*Miscellaneous*	
Strawberries	0	Honey	14
Black raspberries	0	Black strap molasses	17
Red raspberries	0	Vitab	25
Grapefruit	0	Streptomycin matt	57
Lemons	9	Non-hopped wort	17
Oranges	9	Hopped wort	11
Orange skins	39	Dry beer solids	11

inactive; cerevisiae brewers' yeasts were quite active (table 1), in spite of the fact that the hopped wort upon which they were grown contained very little XOF. In the processing of yeasts to be used as food, much of the activity was present in the medium used (36 μg Mo/100 gm).

DISCUSSION

The molybdenum concentration in plants is known (Nicholas, '50; Plant, '51; Mulder, '48a, b; Barshad, '48; Ferguson et al., '40) to vary with: (1) the type of plant, (2) the Mo content or the acidity of the soil, or both, and (3) the higher concentration of Mo in the leaves as compared with the stems or roots. Hence no absolute value for the Mo content of foods can be given. This is illustrated in the present series by the values found for two different samples of onions — one of which contained 4 times as much Mo as the other. Nonetheless, it is evident that legumes and cereal grains are generally high in Mo (Barshad, '48), with the Mo in the latter being concentrated to some extent in the germ (Bertrand, '39) and possibly the bran. Legumes and cereals can absorb relatively large amounts of Mo from alkaline soils rich in Mo (Barshad, '48; Robinson and Edgington, '48) and this seems to be related to the essential role of this element in the root nodules for the fixation of nitrogen (Mulder, '48a, b; Jenson, '45). Very little information is currently available about the Mo requirement of cereal grains for growth, except that oats have been reported (Piper, '40) to give low yields on Mo-deficient soils.

The crucifers have been reported (Bertrand, '39) to be rich in Mo and to require Mo for growth. Cauliflower and broccoli plants are subject to a Mo-deficiency disease, whiptail, in which the leaves contain less than one-fifth their normal amount of Mo (Nicholas, '50; Plant, '51). In the assay of the portions usually eaten, crucifer leaves (such as cabbage) were fairly active, while the roots (turnip) or head (cauliflower) were less active. Concentration of Mo in the leaves was also shown for other plants, such as beets. Grasses, such as al-

falfa, clover, cerephyl, and so forth, can vary from less than 0.1 p.p.m. on deficient soils (Mulder, '48a, b; Jenson, '45) to more than 20 p.p.m. on the alkaline "teart" soils which produce Mo toxicity in cattle (Barshad, '48; Ferguson et al., '40).

In the animal, liver, kidney and spleen were richest in Mo; smaller amounts were present in pancreas and duodenum (Bertrand, '43), while muscles contained very little. Following its administration to rats, Mo accumulated in the kidney and intestine (Neilands et al., '48), presumably because of its excretion in urine and feces.

Insofar as the studies overlap and are directly comparable, these results agree in principle with the distribution of Mo previously reported by TerMeulen ('31, '32). TerMeulen found somewhat more Mo in the legumes and somewhat less in the cereal grains, except for corn; the latter may have been low in the present series because it was degerminated, but Robinson and Edgington ('48) also found little or no Mo in corn grown in Virginia. We observed higher levels in potatoes and grasses, and our values for milk agree better with those obtained by Teresi et al. ('42). Such variations are not unexpected, and do not alter the gross pattern of Mo distribution in foods.

SUMMARY

Foods were assayed for available molybdenum by determining the intestinal xanthine oxidase response to the incorporation of the food into an otherwise purified Mo-deficient diet. When the results were compared with the total Mo determined chemically, 50 to 100% of the dietary Mo was usually available for the biological response.

Foods considered to be good sources of Mo (arbitrarily containing more than 0.6 p.p.m. of available Mo on a dry weight basis) included: (1) legumes, (2) cereal grains (3) some dark green leafy vegetables, and (4) liver, kidney and spleen among the animal tissues. Fruits, berries and most root or stem vegetables were relatively poor sources of Mo, inasmuch as

they contained less than 0.1 p.p.m. Brewers' yeasts were good sources of Mo and bakers' yeasts were poor sources, even though the growth media in both cases were not rich in Mo.

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THE UTILIZATION OF CAROTENE

I. AS AFFECTED BY CERTAIN PROTEINS IN THE DIET OF GROWING ALBINO RATS [1, 2]

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In recent years considerable research has been directed toward the study of factors which influence the utilization of carotene and vitamin A. The economic and physiological importance of this field is signified by the estimate that carotene supplies about two-thirds of the vitamin A value of the average human diet in the United States (Food and Nutrition Board, '48); in oriental countries where the consumption of animal products is low, the contribution of carotene is greater.

The level of protein in the diet was found by Dye, Bateman and Porter ('45) to have little effect upon the utilization of vitamin A. On the contrary, the investigations of Baumann, Foster and Moore ('42), Deuel and his associates ('46), Brown and Morgan ('48), Mayer and Krehl ('48) and Moore, Sharman and Ward ('52) indicate connections between vitamin A and dietary protein. Fraps ('46) found that the substitution of casein for corn meal in the diet of albino

[1] Published with the approval of the Director of the Louisiana Agricultural Experiment Station.

[2] Based upon a thesis submitted in August, 1952, by Ibrahim M. ElGindi to the Graduate Faculty of Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] Government of Egypt Scholar. Present address: Faculty of Agriculture, Cairo University, Giza, Egypt.

rats increased the apparent digestibility of carotene and the storage of vitamin A in the liver. Bentley and Morgan ('46) concluded that the character of the vitamin A-deficient basal diet accompanying the ingestion of carotene or vitamin A, rather than the presence or absence of choline, is an important factor in determining the efficiency with which they are utilized. Klosterman et al. ('52) found that when rats were fed equal amounts of carotene or vitamin A, those fed a phosphorus-deficient ration stored larger amounts of vitamin A in the liver. With sheep, they found an inverse relationship between vitamin A blood levels and the intake of inorganic phosphorus. Hove ('52) found that a factor in the lipid fraction of yeast diminished the growth response to beta-carotene. Burns, Hauge and Quackenbush ('51) found that for the utilization of beta-carotene accompanying a basal diet containing 18% casein, the optimum daily dose of alpha-tocopherol was close to 1.0 mg.

In the present experiments, the utilization of carotene as affected by casein, lactalbumin, gluten and zein was studied under conditions which limited the major dietary variables to differences among our specimens of these proteins.

EXPERIMENTAL

Forty-eight male weanling rats (wt., 39 to 48 gm; age, 19 to 24 days) of the Sprague-Dawley strain were selected from 10 litters; two were killed to provide liver controls and the rest were transferred to individual metabolism cages provided with feeders of the type described by Swift et al. ('34) and Forbes et al. ('35). The animals were caged in an air-conditioned room thermostatically controlled at 80°F. They received the U.S.P. vitamin A-deficient test ration for 38 days at the restricted level of 4 gm/rat/day, as described by Koehn ('48). Distilled water was provided ad libitum. None of the rats developed ocular symptoms of any kind. The animals were weighed weekly until the 21st day, and thereafter every three days until the 38th day. Growth usually ceased between the 27th and 30th days. Samples of tail blood were

collected every third day by a technique which incorporated the best features of those described by Casper ('44) and Elliott ('49). The blood was analyzed for vitamin A by the micro method of Bessey et al. ('46). The precautions of Bieri and Schultze ('51) were observed; contrary to their experience, we had no difficulty in easily and satisfactorily sealing the capillary collection tubes [4] with Pyseal cement.

Two of the depleted animals were killed as liver controls. The remaining 44 were divided on the basis of weight into 4 uniform groups, each containing one representative from each of the 11 litter-fours. For 6 weeks [5] each group received one of the 4 test rations. The nitrogen, gross energy, and phosphorus [6] contents of the ration constituents were determined respectively by the Kjeldahl, Emerson bomb calorimeter and Association of Official Agricultural Chemists volumetric methods. The formula of ration 1 was that of the U.S.P. XII vitamin A-deficient test diet. By means of an algebraic computation based on the nitrogen and energy values of the protein, starch and oil, the other rations were so compounded that they were isocaloric and isonitrogenous with ration 1; it is fortuitous that these rations were approximately isophosphoric. Table 1 gives the analytical values and the proportions of the nutrients in the rations.

The gross energy of these rations was 4.15 Cal./gm; N content, 3.17%; P content,[7] ration 1, 0.58%; 2, 0.53%; 3, 0.51%; 4, 0.51%; and 4a, 0.49%. Groups 1, 2 and 3 of the

[4] We used tubes 10 cm long, 2 mm bore, 0.2 mm wall, and open at both ends to collect the blood samples.

[5] For each group of 11 rats, the average duration of the repletion period was 43.3 days. Three of the litter-fours (12 rats) received the repletion rations for 44 days and the carotene supplement for 43 days; 8 of the litter-fours (32 rats) received the repletion rations for 43 days and the carotene supplement for 42 days.

[6] The report of Klosterman et al. ('52) appeared several months after we completed our experiments. By that time, specimens of our compounded rations and of the cornstarch and cottonseed oil used in these rations were not available for phosphorus analysis.

[7] These values do not include any phosphorus contributed by the cornstarch and cottonseed oil.

rats received, respectively, rations 1 (casein), 2 (lactalbumin), and 3 (gluten); group 4 received ration 4a (zein) for two days and thereafter ration 4, the protein of which was 81% zein and 19% casein (cf. Kligler and Krehl, '50). To each rat a daily dose of 21 μg beta-carotene and 1 mg alpha-

TABLE 1

Analytical values of the nutrients and compositions of the rations

	GROSS ENERGY	% N	% P	GRAMS NUTRIENT PER KILOGRAM RATION				
				Ration				
				1	2	3	4	4a
	Cal./gm							
Devitaminized casein	5.37	13.67	0.67	180.0	none	none	36.0	none
Devitaminized lactalbumin	5.17	12.82	0.35	none	192.1	none	none	none
Gluten	5.40	13.03	0.25	none	none	188.9	none	none
Zein	4.85	12.96	0.17	none	none	none	152.1	190.1
Salt mixture, U.S.P. No. 2	...	....	8.42	40.0	40.0	40.0	40.0	40.0
U.S.P. brewers' yeast [1]	4.64	8.04	1.52	80.0	80.0	80.0	80.0	80.0
U.S.P. cornstarch	3.62	0.1	...	650.0	634.8	644.9	630.5	625.6
U.S.P. cottonseed oil	9.27	...	...	50.0	53.1	46.2	61.4	64.3

[1] A vitamin D content of 5 U.S.P. units per gram of each ration was obtained by incorporating 0.277 gm of irradiated yeast (18,000 units vitamin D/gm) in each kilogram of ration.

tocopherol was administered by the method of Koehn ('48).

During the first 9 days of the repletion period, the average consumption of rations 1, 2 and 3 was about 10 gm/rat/day, while that of ration 4 was 8 gm. These amounts of fresh rations were offered daily thereafter. Individual records were kept of all feed refusals. The average amounts (grams/rat/day) actually consumed were: ration 1, 9.76 ± 0.02 [2]; 2,

[2] Numbers after ± are standard error of the mean.

9.47 ± 0.10; 3, 9.26 ± 0.15; and 4, 6.42 ± 0.27. The rats were weighed weekly.

Collections of blood were made at 4 and 8 hours after the initial dose of carotene, 8 hours after the second dose, 16 hours after the third dose and, thereafter, every three days about 16 to 20 hours after the administration of carotene.

The feces from each rat were quantitatively collected during the repletion period and stored at 0°F. until analyzed for carotene by the method of van Eekelen, Engel and Bos ('42). The rats were anaesthetized with chloroform, and the liver was removed and blotted with heavy filter paper to remove adhering blood. It was then weighed in a closed glass container and stored at 0°F. until analyzed for vitamin A by the method of Vavich and Kemmerer ('50).

RESULTS

The characteristics of the weanling rats were: body wt., 43.8 ± 0.3 gm; blood vitamin A, 24.8 ± 0.9 μg/100 ml serum; liver vitamin A, 7 and 10 μg/liver for the two controls. The finding of only small amounts of hepatic vitamin A is in agreement with the observation of Guerrant ('49) that the liver of the newly-born rat contains very little vitamin A, and that only limited storage occurs during the nursing period.

The average body weights and blood vitamin A levels of the depleted rats composing the 4 matched groups were respectively: group 1, 62.3 ± 1.0 gm, 11.1 ± 1.4 μg; group 2, 62.3 ± 0.8 gm, 11.5 ± 1.6 μg; group 3, 62.3 ± 0.6 gm, 10.5 ± 1.5 μg; group 4, 62.3 ± 0.7 gm, 11.9 ± 1.2 μg. No vitamin A was found in the livers of the two depleted controls. During the last week of the depletion period, the blood vitamin A level of one of these controls was steady at 12 to 14 μg/100 ml serum, while the values of the other control fluctuated between 18 and 25 μg. These results agree with the report of Glover, Goodwin and Morton ('47) that blood vitamin A levels are maintained near normal even when liver stores approach exhaustion.

TABLE 2

Characteristics of the repleted rats

(Average/rat/6 weeks, based on groups of 11 rats)

RATION AND GROUP NO.	1 (Casein)	2 (Lactalbumin)	3 (Gluten)	4 (Zein-casein)	F [1]	LSD [2]
Ration intake; gm	422.5 ± 1.3	409.8 ± 4.3	400.7 ± 6.5	277.9 ± 11.5		
Wt. feces; gm	15.65 ± 0.40	26.40 ± 0.66	24.14 ± 0.71	16.23 ± 0.70		
Final body wt.; gm	206.8 ± 2.0	198.3 ± 3.8	166.9 ± 2.7	126.2 ± 5.3		
Liver wt.; gm	7.47 ± 0.21	8.46 ± 0.24	7.03 ± 0.21	5.76 ± 0.35		
Fecal carotene; μg	191.0 ± 19.6	282.3 ± 11.8	254.5 ± 7.6	162.3 ± 20.8	12.1 [5]	64.2
Hepatic vitamin A; μg/liver	68.7 ± 6.0	35.4 ± 5.4	40.5 ± 4.6	12.1 ± 2.7	23.1 [5]	19.4
Unaccounted for carotene [3];						
(a) μg,	632 ± 19	572 ± 11	595 ± 8	714 ± 21	15.7 [5]	64
(b) % of total ingested	71.2 ± 2.1	64.4 ± 1.2	67.0 ± 0.9	80.4 ± 2.4		7.2
(Liver wt./body wt.) × 100	3.61 ± 0.08	4.29 ± 0.17	4.21 ± 0.11	4.55 ± 0.18	7.9 [5]	0.56
Growth efficiency [4]	0.342 ± 0.004	0.332 ± 0.006	0.261 ± 0.005	0.226 ± 0.011	65.1 [5]	0.028

[1] F = F value as calculated by analysis of variance. At the 1% point, F = 4.31.

[2] LSD = least significant difference (P = 0.05).

[3] The unaccounted for carotene is the difference between the amount of carotene ingested and the sum of the amounts representing the fecal carotene and the hepatic vitamin A.

[4] The unexpected failure of lactalbumin to promote better growth than casein is probably due to changes which occurred in the lactalbumin during the devitaminizing process (*cf.* Davis, Rizzo and Smith, '49; Schroeder, Stewart and Smith, '51). According to the manufacturer who donated the casein and lactalbumin for experimental purposes, both were devitaminized by the same process.

[5] Significant at the 1% level of probability.

The terminal data pertaining to the repleted animals are given in table 2.

These data show that smaller amounts of fecal carotene are associated with casein and zein-casein than with lactalbumin and gluten; that the liver stores of vitamin A associated with casein are 5 times those observed with zein-casein, and almost twice those found for lactalbumin and gluten; that, relative to the size of the body, livers associated with casein are about 20% smaller than those associated with the other proteins; that the amount of unaccounted for carotene associated with zein-casein is greater than that associated with the other proteins; and, as has long been known, that the growth efficiencies of casein and lactalbumin are far superior to those of gluten and zein.

Approximately 1,300 samples of blood were collected and analyzed for vitamin A. Statistical evaluation of the results showed no significant difference in blood vitamin A among the 4 groups during the depletion period. The values increased from about 25 to 35 μg vitamin A/100 ml serum during the first two weeks, when the growth of the rats was relatively good on the restricted intake of the U.S.P. vitamin A-deficient test ration. During the next two weeks the values decreased to about 11 μg vitamin A/100 ml serum, where they remained essentially constant. The onset of the constant low values approximately coincided with the cessation of growth. The values quadrupled to about 45 μg within 4 hours following the initial dose of carotene (cf. Thompson, Ganguly and Kon, '49). This high level persisted for about a week. During the following month, the values gradually decreased to about 32 μg vitamin A/100 ml serum. Based upon groups of 11 rats each, the statistical results indicated that the blood vitamin A levels associated with casein and lactalbumin approached, but rarely attained, values which were significantly higher than those associated with gluten and zein-casein. On the basis of two groups of 22 rats each — one group being composed of the rats which received the milk proteins of good over-all amino acid balance, and the other group being

composed of the rats which received the cereal proteins of poor over-all amino acid balance — the statistical results showed, at the 5% probability level, a significant difference which averaged about 17% higher for the milk proteins from the third to the 30th day. After the 30th day, when the rats were relatively mature, no significant difference was observed between the blood vitamin A levels of the two groups.

DISCUSSION OF RESULTS

The fate of the ingested carotene which was not excreted in the feces or stored in the liver as vitamin A is unknown. The unaccounted for carotene would comprise (a) that consumed in metabolic processes, (b) any carotene or vitamin A which may have been stored in tissues other than the liver, and (c) any carotene destroyed in the intestinal tract. Sexton, Mehl and Deuel ('46) reported that no carotene was destroyed in the excised gastrointestinal tract of rats, kept at 37°C. for 24 hours. In the present experiments, the fact that vitamin A was stored in the liver of each rat suggests that the animals converted carotene to vitamin A in amounts sufficient to satisfy all physiological needs. The minimum value of the intake of vitamin A necessary for these needs — that is, the daily amount of vitamin A adequate for the good growth and general well-being of the rat but permitting little or no storage — has been variously reported by different investigators (cf. Paul and Paul, '46). That these physiological needs may vary, depending upon the type of protein in the diet, is suggested by the finding of significantly greater amounts of unaccounted for carotene associated with the zein-casein ration.

The antithetical patterns of the data in table 2 suggest that vitamin A is consumed in a liver process which transforms moieties from the ingested protein to other proteins or amino acids which the body tissues can utilize. In the rationalization of such a process, from a protein associated with poor growth efficiency a greater proportion of moieties would have to be transformed by the liver and the greater would be the size

of the liver relative to that of the body; concomitantly, the consumption of vitamin A would be increased, thus leaving a smaller amount of vitamin A to be stored in the liver. Conversely, the fact that the largest amounts of vitamin A were found in the livers which were smallest relative to the size of the body may be explained in a similar way. The protein in the ration of highest efficiency (casein, under the conditions of our experiments) furnished moieties, of the kinds and in the proportions, corresponding more nearly to those which are readily acceptable to the body tissues. The transforming, or converting, demands on the liver would be less with this ration. In consequence, the size of the liver relative to that of the body would be smaller; the consumption of vitamin A would be less, and a larger amount of vitamin A would remain for storage in the liver.

Interpretation of the blood vitamin A results in terms of the concept of plasma carrier proteins, recently advanced by Ganguly, Krinsky, Mehl and Deuel ('52), suggests that the rations containing the milk proteins furnished a greater proportion of the moieties which produce the vitamin A carrier proteins than did those containing the cereal proteins.

SUMMARY

Comparisons were made of some effects of casein, lactalbumin, gluten and zein in isocaloric, isonitrogenous and essentially isophosphoric rations on the nutritional utilization of beta-carotene by growing albino rats.

Rats which received diets containing lactalbumin or gluten excreted more carotene in the feces than did those which received diets containing casein or zein. The rat livers associated with casein were about 20% smaller, relative to the size of the body, than the livers associated with the three other proteins, yet these smaller livers stored two to 5 times as much vitamin A as did the livers associated with lactalbumin, gluten or zein when the carotene intakes were the same for all 4 groups. Rats which received casein or lactalbumin had blood vitamin A levels about 17% higher than those of the ani-

mals which recived gluten or zein. Regardless of the protein in the diet, the blood vitamin A levels of young, rapidly growing rats were about 40% higher than those observed a few weeks later when the animals had attained maturity.

The antithetical aspects of these findings suggest that vitamin A is consumed in a liver process which transforms moieties from the ingested protein to other proteins or amino acids.

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QUANTITATIVE VALINE REQUIREMENT OF THE WEANLING PIG [1]

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The amino acids first found to be required by swine were those which are present in relatively small amounts in certain natural proteins or in special hydrolysates. Using diets containing such proteins or protein hydrolysates, it was possible to demonstrate the essential nature or the quantitative requirement, or both, of the 5 amino acids tryptophan (Beeson et al., '48; Shelton et al., '51a), lysine (Mertz et al., '49; Brinegar et al., '50b; Shelton et al., '51b), methionine (Bell et al., '50; Shelton et al., '51c; Curtin et al., '52b), isoleucine (Brinegar et al., '50a), and histidine (Loosli, '51). The effect of other amino acids remained unknown until purified amino acid diets were introduced by this laboratory and were used to demonstrate that, for good growth, the weanling pig requires only 5 additional amino acids, namely, threonine (Shelton et al., '50), and arginine, leucine, phenylalanine and valine (Mertz et al., '52). In this group of amino acids only threonine has been quantitated (Beeson et al., '53). The threonine quantitation was carried out at this laboratory, using a new approach in diet formulation, i.e., feeding a natural feedstuff which provides a low protein level and building from it a bal-

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anced protein at an adequate level with the 10 essential amino acids and diammonium citrate. This technique can be adapted to measurement of the requirement for other amino acids, and was so used with respect to valine, with the results reported here.

EXPERIMENTAL PROCEDURE

Major components of the basal diet included corn, 10 essential amino acids, diammonium citrate, minerals, liver extract and cerelose (table 1). This diet had a total crude protein equivalent of 12.8%, a digestible crude protein equivalent of 11.2%, and a digestible L-valine content of 0.3%. Valine was supplied at higher levels by using DL-valine to replace part of the cerelose and diammonium citrate. The diet with the most valine contained no diammonium citrate. The same batch of corn (digestibility 78%) used in the threonine measurement experiments (Beeson et al., '53) was used in these experiments. The amino acid composition of the corn (determined by the microbiological method of Steele et al., '49), the level of amino acids added, the form in which they were used, and the total utilizable amino acids in the diet are shown in table 2. In these studies it was assumed that the amount of amino acid which can be utilized is that present in the digestible protein of the corn, plus the amount of crystalline amino acid which is physiologically active for the rat as described by Rose et al. ('49). The D-form is inactive except for methionine, phenylalanine and tryptophan.

Thrifty crossbred pigs (5 to 6 weeks of age) from a commercial pig hatchery were used in these experiments. The 15 barrows averaging 28.1 lb. were distributed among 5 lots on the basis of initial weight. The pigs were housed in individual concrete pens and fed ad libitum during the 35-day experiment. Necessary vitamin supplements were mixed with the feed daily. The L-valine levels tested were 0.3, 0.4, 0.5, 0.6 and 0.7% of the total diet.

RESULTS AND DISCUSSION

Since L-valine is the only valine isomer which influences the growth of rats (Rose, '38), we have assumed that this is also true for the growth of swine. For this reason further discussion of valine will refer only to L-valine.

In growth trials preliminary to the swine experiments, male rats had a valine requirement of 0.6% of the corn-amino acid

TABLE 1

Composition of basal diet used to determine the valine requirement of the weanling pig

COMPONENTS	AMOUNT
	%
Corn [1]	88.00
Ten essential amino acids [2]	5.64
Diammonium citrate	0.77
Minerals [3]	4.30
Liver extract	0.50
Cerelose	0.79
	100.00 [4]

[1] Contained 8.2% crude protein and 14.1% moisture by analysis (Association of Official Agricultural Chemists, '45).

[2] For composition see table 2.

[3] Beeson et al. ('53).

[4] Crystalline terramycin·HCl (10 mg/lb. feed) and crystalline B_{12} (10 μg/lb. feed) were added to all diets. Vitamin supplements used previously (Mertz et al., '52) were fed daily.

diet. This compares favorably with the value of 0.7% reported by Rose ('37) in studies with mixtures of purified amino acids.

In the swine experiments the corn-amino acid diets produced gains which were highly satisfactory for pigs of this size and age. The daily gains were approximately 1.0 lb. for all valine levels except the lowest (daily gain: 0.67 lb.). Data summarized in table 3 show that 0.3% L-valine was inadequate for optimum growth, while diets containing 0.4 to 0.7% L-valine all gave essentially the same growth. These data (table 3) also indicate that feed intake and feed per pound of gain dif-

fered markedly between pigs fed 0.3% L-valine and those fed 0.4% L-valine, while pigs fed higher levels of valine showed essentially the same results as those at the 0.4% level. The high quality of the protein in the diets containing adequate L-valine was demonstrated by a protein efficiency of 3.2 to

TABLE 2

Amino acid components of the basal diet used to determine the valine requirement of the weanling pig

AMINO ACID	AMINO ACID ASSAY VALUE OF CORN	DIGESTIBLE AMINO ACID IN THE DIET SUPPLIED BY CORN [1]	ADDED CRYSTALLINE AMINO ACID IN THE DIET	FORM OF AMINO ACID USED	TOTAL UTILIZABLE AMINO ACID [2]		
					In diet	In the digestible crude protein of diet [3]	
	%	%	%		%	%	
Arginine	0.17	0.12	0.10	L·HCl	0.20	1.8	(1.6)
Histidine	0.15	0.10	0.38	L·HCl·H_2O	0.38	3.4	(3.0)
Isoleucine	0.22	0.15	0.70	L with D allo	0.50	4.5	(3.9)
Leucine	0.80	0.55	0.26	L	0.81	7.2	(6.3)
Lysine	0.15	0.10	2.25	DL·HCl	1.00	8.9	(7.8)
Methionine	0.13	0.09	0.51	DL	0.60	5.4	(4.7)
Phenylalanine	0.26	0.18	0.72	DL	0.90	8.0	(7.0)
Threonine	0.29	0.20	0.40	DL	0.40	3.6	(3.1)
Tryptophan	..	...	0.20	DL	> 0.20	> 1.8	> (1.6)
Valine	0.35	0.24	0.12	DL	0.30	2.7	(2.3)[4]

[1] Assay value × per cent of corn in diet × per cent digestibility of corn protein, or assay value × 88% × 78%.

[2] Digestible amino acid from corn plus physiologically active amino acid from the crystalline form.

[3] Digestible crude protein = 11.2% of diet. Figures in parentheses denote per cent of utilizable amino acid in total crude protein in the diet. Total crude protein = 12.8% of diet.

[4] At lowest valine level.

3.4 (pounds gain per pound total crude protein). This protein efficiency is greater than the values of 2.1 to 2.6 obtained with high quality natural proteins by other workers (Guerin et al., '51; Catron et al., '52).

According to these results, the L-valine requirement of the weanling pig is 0.4% of the diet, 3.6% of the digestible crude

protein or 3.1% of the total crude protein. From tissue analysis experiments Curtin et al. ('52a) calculated the valine requirement of the pig to be 3.9% of the total crude protein. In these tissue analysis experiments, the lysine requirement served as the base for further calculations. If another essential amino acid were present in the swine tissues at the same level as lysine, it was assumed to be required in the same amount. On this basis, analysis of swine tissues indicated a requirement for valine which is 70% of the lysine requirement,

TABLE 3

Average data[1] *for pigs receiving various levels of valine in the diet*

ADDED DL-VALINE (%)	0.12	0.32	0.52	0.72	0.92
TOTAL UTILIZABLE L-VALINE (%)	0.3	0.4	0.5	0.6	0.7
Initial weight (lb.)	28.2	28.2	28.2	28.0	28.0
Final weight (lb.)	51.5	62.8	63.7	64.5	63.3
Daily gain (lb.)	0.67	0.99	1.01	1.04	1.01
Daily feed consumption (lb.)	1.79	2.40	2.34	2.52	2.30
Feed per pound of gain (lb.)	2.67	2.42	2.32	2.42	2.28
Protein efficiency[2]	2.9	3.2	3.4	3.2	3.4

[1] Three pigs/lot. Duration of experiment, 35 days.

[2] Pounds gain per pound total crude protein.

whereas our feeding experiments indicate a valine requirement which is not more than 40% of the lysine requirement. Thus, placing the lysine requirement at 1.0% of the diet, the tissue analysis data give a value for valine which is much higher than that found by growth experiments. The difference between our value and that of Curtin et al. ('52a) would be reduced somewhat if the D-isomer, inactive for the rat, is equal in activity to the L-isomer for the pig. Then the requirement for valine by our growth experiments would be 56% of the lysine requirement. No data are available, however, on the growth-promoting properties of D-valine in swine.

Tissue analysis experiments can be misinterpreted for an amino acid like arginine, which is synthesized to a limited extent by the weanling pig (Mertz et al., '52). Thus, pigs

show an excellent growth response when the diet contains 0.2% L-arginine, or 20% of the lysine requirement (Mertz et al., '52; Beeson et al., '53). The arginine requirement based on tissue analysis data is about 4 times this value, namely, 83% of the lysine requirement (Curtin et al., '52a).

The utilizable amino acid content of the diet (digestible amino acid from corn plus physiologically active amino acid from the crystalline form) is expressed as "per cent of the diet" and as "per cent of the digestible crude protein" (table 2). The former expression has come from the work of Rose ('37), who determined the amino acid requirements of the rat, using amino acid diets. The amino acid levels expressed as a per cent of the diet in table 2 are essentially the same as those given by Rose et al. ('49) as "physiologically active." The latter expression (per cent of the digestible crude protein) is a modification of the term used by Brinegar et al. ('50b), who represented the amino acid requirements of swine in terms of the total crude protein of the diet. The expression of the requirement of an amino acid with respect to the total crude protein in the diet appears to be a valid one, providing that: (1) the protein is well-balanced with respect to the amino acid requirements of the animal; (2) the total crude protein is not fed in excess of the requirement; and (3) all of the dietary proteins have a high digestibility.

These requirements would be substantially met by a balanced amino acid mixture (fully digestible), or by a natural ration containing a balanced protein of high digestibility. In diets such as those used in the valine experiment in which the dietary "protein" is composed of both incompletely digestible natural proteins and completely digestible crystalline amino acids, the expression of an amino acid requirement as a per cent of the total crude protein would give an incorrect relationship. In this type of diet, the ratio of total amino acid to total crude protein is not the same as the ratio of digestible amino acid to digestible crude protein. With a protein-amino

acid diet, representing the digestible essential amino acid as per cent of the digestible crude protein would be more nearly correct.

SUMMARY

Weanling pigs were grown on corn-amino acid diets of equal nitrogen content which contained sufficient DL-valine to provide utilizable L-valine at levels of 0.3, 0.4, 0.5, 0.6 and 0.7% of the diet. The rate of gain and efficiency of feed utilization were markedly inferior at the 0.3% L-valine level, while the gain and feed utilization were uniformly high when the diet contained 0.4 to 0.7% L-valine. Assuming that D-valine is inactive, these data suggest that the L-valine requirement of the weanling pig is 0.4% of the total diet, 3.6% of the digestible crude protein, or 3.1% of the total crude protein.

The high quality of the protein in the corn-amino acid diets was demonstrated by a protein efficiency of 3.2 at the 0.4% L-valine level.

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SOME FACTORS AFFECTING NITROGEN BALANCE IN THE ADULT RAT

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The experiments described in this paper were undertaken to determine the effect of different levels and sources of dietary nitrogen and the nature of the dietary carbohydrate on nitrogen balance in the adult rat at low levels of amino acid feeding. For this purpose two levels of essential amino acids were fed and the nitrogen intake was varied by incorporating in the rations two levels of diammonium citrate. Preliminary experiments had indicated a difference in nitrogen balances of animals depending upon the nature of the carbohydrate moiety of the ration. Therefore, after the nitrogen output had been determined on a diet containing sucrose, the animals were transferred to similar rations containing corn or potato dextrin.

Three series of experiments were conducted. In the first two, the response of undepleted or protein-depleted adult rats to variations in level and source of nitrogen and in type of carbohydrate was investigated. In the third series, variations in nitrogen balances due to type of carbohydrate in relation to the length of time of amino acid feeding were studied.

EXPERIMENTAL

The compositions of the amino acid mixtures and of the diets employed are given in tables 1 and 2. The non-essential amino acids were incorporated in the rations at a level of 0.1%. The physiologically effective forms of the essential amino ac-

ids were included in the rations at levels of either 0.1 (mixture 1) or 0.2% (mixture 2). Diammonium citrate was added to diets 3 and 4 at levels of 0.73 and 2.50%. When the animals ingested 12 gm of the sucrose-containing rations, diet 2 furnished 37 mg total nitrogen, diet 3 48 mg, diet 4 72 mg, and diet 5 48 mg. Diets 2, 3 and 4 supplied the same amounts of essential amino acids with three levels of total nitrogen; diets 3 and 5 furnished equal amounts of total nitrogen with two levels of essential amino acid nitrogen. In order to keep the nitrogen intake on diet 5 equal to that of diet 3 without decreasing the caloric intake, 10.8 gm of diet 5 and 1.2 gm per day of the nitrogen-free ration (diet 1) were fed, and thereby the daily allotment of each of the non-essential amino acids was decreased to 10.8 mg, as compared with 12.0 mg received by the animals ingesting diets 2, 3 and 4. This small decrease is believed to be without bearing on the results of the study. Animals ingesting 12 gm of the rations containing dextrin received about 5 mg more nitrogen per day than the amounts furnished in the sucrose rations. Since information was lacking as to the character of this nitrogen, it was counted only as total nitrogen.

Male rats from the laboratory stock colony [1] were housed individually and fed a commercial pelleted diet from weaning until the start of the experiments. The laboratory was air-conditioned, with the temperature maintained at $80 \pm 2°$F. In series 1, 36 animals 70 to 79 days old, weighing 265 to 357 (ave. 302) gm, were placed directly upon experimental diets 2 to 5, without prior depletion of their nitrogen stores. From 8 to 10 animals were placed on each diet, the several groups being equalized as closely as possible according to litter and body weight. They were fed ad libitum for 4 days to allow for adjustment to the experimental rations, after which weighed amounts of the rations were offered daily for 8 weeks and urine and fecal collections were made for each 7-day period.

[1] A purebred strain resulting from the crossing of albino and black and white hooded strains of *mus norvegicus*. The litters may include white, black, and black and white rats.

During the first 4 weeks sucrose was the carbohydrate in the rations, and in the final 4 weeks corn dextrin was substituted for sucrose. The first period in which the animals received corn dextrin was considered to be an adjustment period and is not included in any evaluation of the data.

TABLE 1

Composition of amino acid mixtures

CONSTITUENTS	MIXTURE 1		MIXTURE 2	
	Physiologically active	As used	Physiologically active	As used
	gm	*gm*	*gm*	*gm*
DL-alanine	0.1	0.1	0.1	0.1
L-arginine	0.1		0.1	
L-arginine HCl		0.12		0.12
L-aspartic acid	0.1	0.1	0.1	0.1
L-cystine	0.1	0.1	0.1	0.1
L-glutamic acid	0.1	0.1	0.1	0.1
Glycine	0.1	0.1	0.1	0.1
L-histidine	0.1		0.2	
L-histidine·HCl·H_2O		0.135		0.27
L-hydroxyproline	0.1	0.1	0.1	0.1
DL-isoleucine	0.1	0.2	0.2	0.4
L-leucine	0.1	0.1	0.2	0.2
L-lysine	0.1		0.2	
L-lysine HCl		0.125		0.25
DL-methionine	0.1	0.1	0.2	0.2
DL-phenylalanine	0.1	0.1	0.2	0.2
L-proline	0.1	0.1	0.1	0.1
DL-serine	0.1	0.1	0.1	0.1
DL-threonine	0.1	0.2	0.2	0.4
DL-tryptophan	0.1	0.1	0.2	0.2
L-tyrosine	0.1	0.1	0.1	0.1
DL-valine	0.1	0.2	0.2	0.4
$NaHCO_3$		0.160		0.27
	1.9	2.440	2.8	3.81

In series 2, 48 rats 91 to 106 days old, weighing 307 to 388 (ave. 341) gm, were depleted of their protein stores by feeding them ad libitum for 18 days a nitrogen-free ration (diet 1) containing sucrose. At the end of this time, when their body weights varied from 270 to 328 (ave. 292) gm, the ani-

mals, distributed into groups of 12 according to litter and body weight, were placed on diets 2 to 5 containing sucrose as the carbohydrate. After three weeks the source of the carbohydrate was changed and the experiments continued for

TABLE 2

Composition of diets[1]

CONSTITUENTS	DIET				
	1	2	3	4	5
	gm	gm	gm	gm	gm
Amino acid mixture 1		2.44	2.44	2.44	
Amino acid mixture 2					3.81
Diammonium citrate			0.73	2.50	
Carbohydrate[2]	80.65	78.21	77.48	75.71	76.84
Salt mixture[3]	4.00	4.00	4.00	4.00	4.00
Corn oil[4]	3.00	3.00	3.00	3.00	3.00
Lard	12.00	12.00	12.00	12.00	12.00
Vitamin A and D concentrate[5]	0.05	0.05	0.05	0.05	0.05
Inositol	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.20	0.20	0.20	0.20	0.20
	100.00	100.00	100.00	100.00	100.00

[1] The following vitamins were added per kilogram ration:

Thiamine HCl, 5 mg; pyridoxine HCl, 5 mg; nicotinic acid, 5 mg; riboflavin, 10 mg; Ca pantothenate(d), 25 mg; *p*-aminobenzoic acid, 300 mg; α-tocopherol acetate, 25 mg; 2-methyl-1,4-naphthoquinone, 2 mg; biotin, 100 μg; folic acid, 2 mg; vitamin B_{12}, 30 μg.

The caloric contents of the diets were calculated to be as follows:

4.4 cal. per gram: diet 4, sucrose.

4.5 cal. per gram: diet 1, sucrose; diet 2, sucrose; diet 3, sucrose; diet 4, dextrin; and diet 5, sucrose.

4.6 cal. per gram: diet 2, dextrin; diet 3, dextrin; diet 5, dextrin.

[2] Sucrose, diets 1 to 5; corn dextrin, diets 1 to 5; potato dextrin, diets 2 and 5.

[3] Jones and Foster ('42).

[4] Mazola.

[5] Squibb's Navitol containing 65,000 U.S.P. units of vitamin A and 13,000 units of vitamin D per gram.

three additional weeks. One-half of the animals previously fed diets 2 and 5 containing sucrose received corn dextrin as the source of carbohydrate, while the remainder received potato dextrin. Six of the animals of diet 3 were given corn dextrin and the others received corn dextrin plus 1% sulfa-

suxidine. One-half of the animals receiving diet 4 were changed from sucrose to corn dextrin and the others were used in another study, the results of which are not reported here. The first week of each three-week period was used as an adjustment period before collection of urine and feces.

In series 3, 21 male rats 90 to 99 days old, weighing 288 to 362 (ave. 324) gm, were depleted by feeding the nitrogen-free diet 1 containing sucrose (groups A and B) or corn dextrin (group C) ad libitum for 18 days, at which time their body weights varied from 251 to 306 (ave. 275) gm. The 7 animals comprising group A received diet 2 containing amino acid mixture 1 and sucrose for 6 weeks. The 8 animals in group B were given diet 2 containing sucrose for three weeks and then placed on diet 2 containing corn dextrin for three weeks. The 6 animals of group C received diet 2 containing corn dextrin for three weeks from the end of the nitrogen-free period. No collections of urine or feces were made during the first week after the nitrogen-free period.

Except where otherwise indicated, all animals were offered 12 gm of diet each day. The rats and the uneaten food were weighed weekly. If food was scattered, or if the essential amino acid nitrogen intake fell below 13.0 mg per day on diets 2, 3 and 4, or below 23.0 mg on diet 5, in series 1 and 2, the data from those periods were discarded. In series 3, the food intakes of the animals were in general lower than those in the first two experiments and all periods except those in which scattering occurred are included in the averages.

During the periods when nitrogen balances were determined, the animals were placed in wire metabolism cages with one-half-inch mesh bottoms supported above 250-mm glass funnels. Under the funnels were glass bottles containing toluene and sufficient sulfuric acid to acidify the urine samples. Fine mesh screens over the tops of the funnels retained the feces, which were collected daily and placed in concentrated sulfuric acid. Two or three times during each period the feces in sulfuric acid were partially digested to prevent the foaming which occurred if the entire sample was digested at once.

One per cent ferric oxide was added to the food at the beginning of each 7-day period to serve as a feces marker. Several times during each period the screens and funnels were washed down with a fine stream of hot 2% boric acid solution, this procedure again being employed at the end of the period when the animals were transferred to clean cages. The used cages were washed in a similar manner, and all washings added to the weekly urine collections. The further treatment of the urine and feces has already been described (Womack, Harlin and Lin, '53). Analysis of variance was used to aid in evaluation of the results.

RESULTS AND DISCUSSION

Increasing the average daily total nitrogen intake of the undepleted animals from 35.8 to 46.4 or 72.6 mg by the addition of diammonium citrate without change in essential amino acid nitrogen caused small but not statistically significant changes in the negative nitrogen balances, from — 23.2 to — 19.4 to — 17.2 mg (table 3, series 1). When the total nitrogen intake was raised to 47.7 mg by increasing the essential amino acid nitrogen intake to 25.1 mg per day, the average nitrogen balance of — 7.2 was significantly better than that of — 19.4 for the animals receiving 46.4 mg of total nitrogen but only 14.5 mg of essential amino acid nitrogen (diet 3). It is apparent that 12 gm per day of the sucrose ration containing amino acid mixture 1 do not meet the essential amino acid requirement of adult rats. The nitrogen of the diammonium citrate appears to have little influence on the nitrogen balances of undepleted animals when added to rations containing these levels of amino acids. These results were confirmed in a group of animals partially depleted of protein (table 3, series 2).

Comparison of the nitrogen balances of the animals receiving diets 2, 3 and 4 containing sucrose revealed that there were greater nitrogen losses in the undepleted (series 1) than in the depleted animals (series 2) at comparable levels of total and essential amino acid nitrogen intake. This is to be

TABLE 3

Average daily nitrogen balance data for undepleted (series 1)[1] or protein-depleted (series 2) adult male rats receiving rations containing low levels of amino acids with and without supplementation with diammonium citrate and with either sucrose, corn dextrin or potato dextrin

SERIES NO.	CARBOHYDRATE OF RATION	DIET NO.	INITIAL WEIGHT	FINAL WEIGHT	NO. OF 7-DAY PERIODS	INTAKE				NITROGEN EXCRETION		NITROGEN BALANCE
						Food	Calories	Total nitrogen	E.A.A.N.[2]	Feces	Urine	
			gm	*gm*		*gm*		*mg*	*mg*	*mg*	*mg*	*mg*
1	Sucrose	2	292[3]	278	30	11.9	54	35.8	14.7	12.8	46.2	— 23.2
		3	284[3]	271	38	11.8	53	46.4	14.5	12.0	53.8	— 19.4
		4	281[3]	271	34	11.8	52	72.6	14.5	12.3	77.5	— 17.2
		5	294[3]	281	31	11.9	54	47.7	25.1	12.4	42.5	— 7.2
	Corn dextrin[4]	2	282	287	13	11.8	54	41.4	14.5	14.4	30.6	— 3.6
		3	271	281	21	11.6	53	50.3	14.3	13.5	35.1	+ 1.6
		4	272	276	12	11.4	51	74.2	14.0	13.7	61.9	— 1.3
		5	283	297	21	11.9	55	52.7	24.9	14.8	34.7	+ 3.2
2	Sucrose	1	341	292	47	9.8	44	2.0		10.5	52.0	— 60.6
		2	291	296	23	11.9	53	36.2	14.6	11.9	37.0	— 12.7
		3	293	294	21	11.7	52	45.7	14.4	11.4	45.7	— 11.3
		4	294	290	18	11.8	52	71.3	14.5	12.9	70.9	— 12.5
		5	292	298	18	11.8	53	45.0	24.7	11.3	36.7	— 3.0
	Corn dextrin[4]	2	302	302	11	11.7	53	41.1	14.4	14.5	30.2	— 3.6
		3	301	306	9	11.5	52	49.5	14.1	14.9	36.6	— 2.1
		4	297	297	12	11.7	52	75.8	14.3	14.2	60.9	+ 0.7
		5	305	307	12	11.8	54	52.0	24.7	14.4	35.2	+ 2.4
	Potato dextrin[5]	2	293	292	10	11.9	54	41.8	14.7	23.3	28.0	— 9.5
		5	286	290	12	12.0	54	51.4	25.0	21.6	31.9	— 2.1
	Corn dextrin with 1% sulfasuxidine	3	288	296	10	11.6	52	64.0	14.3	26.9	32.4	+ 4.7

[1] Two animals from each group were killed at the end of the period of sucrose feeding.
[2] Essential amino acid nitrogen.
[3] Weight after 4-day adjustment period. Losses during that period: diet 2, 16 gm; diet 3, 17 gm; diet 4, 18 gm; diet 5, 8 gm.
[4] Amidex, from Corn Products Refining Co.
[5] From Morningstar, Nicol, Inc.

expected, since undepleted animals apparently have large stores of labile protein to call upon when their amino acid requirements are not met by intake. It might be expected that over a period of a month on low nitrogen rations the protein reserves would be gradually reduced and the nitrogen balances of the undepleted would approach those of the depleted group. This is not the case, however, as is shown by average nitrogen balances of — 25.2, — 23.8, — 22.7 and — 20.9 mg per day for 4 successive 7-day periods for the animals of series 1, diet 2, on which the largest decrease was observed. Apparently the amino acid intake met a large enough proportion of the requirement so that the body stores were not approaching depletion even at the end of the 4th week on the ration.

When the animals of series 1 and 2 were changed from rations containing sucrose to those containing corn dextrin, some unexpected results were obtained. Nitrogen losses were lower in all groups receiving amino acid mixture 1, and the balances of the animals receiving diets containing either mixture 1 or mixture 2 were not significantly different from zero. The effect on nitrogen balance of a change from sucrose to corn dextrin, therefore, was approximately equivalent to an increase in essential amino acid intake. In this study there were no statistically significant differences among the balances for the animals on any of the rations containing corn dextrin, nor between the depleted and undepleted animals on those rations. There were, however, statistically significant differences between the values for each pair of rations containing sucrose or corn dextrin. When 1% sulfasuxidine replaced an equal amount of corn dextrin in diet 3, the average daily nitrogen balance of + 4.7 mg was the highest obtained on any ration, and was significantly higher than the value of — 2.1 obtained for litter mates receiving diet 3 and corn dextrin but no sulfasuxidine. The difference between the balances for the animals receiving sucrose or potato dextrin is significant, but is overshadowed by the much greater improvement brought about by the change to corn dextrin (diet 2,

series 2). The difference between the balances of the animals receiving sucrose or potato dextrin and the higher levels of essential amino acids (diet 5, series 2) is not significant, while the change to corn dextrin results in a significant improvement in the balances.

The largest increases in nitrogen intake resulting from the change to corn dextrin were from 35.8 to 41.4 mg per day for the animals of series 1, diet 2, which was associated with a significant change in nitrogen balance from — 23.2 to — 3.6; and from 45.0 to 52.0 mg per day for the animals of series 2, diet 5, which was associated with a significant change in nitrogen balance from — 3.0 to + 2.4. In the latter instance it is possible that the change in nitrogen balance of 5.4 mg can be accounted for by the increase in intake of 7.0 mg. In the former case, however, it seems unlikely that an increase in intake of 5.6 mg would improve the nitrogen balance by 19.6 mg. Moreover, increases in the nitrogen intake of many times that amount by the addition of diammonium citrate brought about no such changes in nitrogen balances on the sucrose-containing rations. Also, in the undepleted animals of series 1, diet 4, there was an increase in average nitrogen intake of only 1.6 mg and yet the nitrogen balances rose from — 17.2 to — 1.3 mg per day.

Initial and final weights of the animals of series 1 and 2 are included in table 3. Except for the losses during the periods when nitrogen-free rations were fed, the only appreciable variations in weight were shown by the undepleted animals of series 1 during the periods of sucrose feeding.

The nitrogen balances of the protein-depleted animals of series 3 are presented in table 4. Although the effects on nitrogen balance of small differences among the groups in the food, calorie, total nitrogen and essential amino acid nitrogen intake cannot be evaluated, it seems clear that the improved nitrogen balances are due to the change of the carbohydrate to corn dextrin and not to a gradual change in the balances when the animals remain on the rations containing sucrose for relatively long periods of time.

TABLE 4

Average daily nitrogen balance data for protein-depleted adult male rats (series 3)[1] receiving rations containing low levels of amino acids (diet 2) and with either sucrose or corn dextrin

GROUP NO.[1]	CARBOHYDRATE OF RATION	WEEK OF AMINO ACID FEEDING	INTAKE				NITROGEN EXCRETION		NITROGEN BALANCE
			Food	Calories	Total nitrogen	E.A.A.N.[2]	Feces	Urine	
			gm		*mg*	*mg*	*mg*	*mg*	*mg*
A	Sucrose	2	11.0	49	34.6	13.5	11.0	37.6	— 14.1
B	Sucrose	2	11.0	49	34.9	13.6	10.4	37.7	— 13.3
C	Corn dextrin	2	11.3	51	39.5	13.9	14.8	28.3	— 3.7
A	Sucrose	3	11.3	50	35.3	13.8	10.2	37.6	— 12.4
B	Sucrose	3	11.1	50	34.9	13.7	10.6	36.5	— 12.2
C	Corn dextrin	3	11.8	53	40.7	14.5	14.6	30.5	— 4.3
A	Sucrose	4	10.6	47	32.6	13.0	9.7	33.1	— 10.1
B	Corn dextrin[3]	4	11.0	50	37.3	13.5	12.5	30.3	— 5.6
A	Sucrose	5	10.6	48	32.9	13.1	9.3	33.8	— 10.2
B	Corn dextrin[3]	5	11.2	51	38.2	13.8	13.6	29.3	— 4.6
A	Sucrose	6	10.2	46	31.6	12.5	8.8	33.2	— 10.8
B	Corn dextrin[3]	6	11.3	52	39.6	13.9	14.2	28.8	— 3.5

[1] Average starting weights after protein-depletion: group A, 276 gm; group B, 273 gm; group C, 277 gm. Average final weights after amino acid feeding: group A, 270 gm; group B, 276 gm; group C, 270 gm.

[2] Essential amino acid nitrogen.

[3] These animals were fed sucrose for the first three weeks of amino acid feeding.

There are several possible explanations for the effect of dextrin in the ration on the utilization by the adult rat of low levels of amino acids. The dextrin may provide small amounts of either additional essential or non-essential amino acids; the small increase in caloric content of the dextrin diet may be beneficial to the animal; the possibility of the existence of a different type of bacterial flora when the dextrin is present could conceivably affect the availability of dietary amino acids to the host organism; some difference between the physical states of sucrose and dextrin may produce a delay in the elimination of the intestinal contents, thereby making the amino acids available for absorption for a longer time; or a delay in absorption of the dextrin or its digestive products may furnish energy for intestinal synthesis of additional essential or non-essential amino acids. Whether or not such synthesized amino acids, if formed, would be available to the animal is a much-debated question. Although the animals were housed in cages with large mesh bottoms, undoubtedly some feces were consumed, so that amino acids formed might be available in this fashion even if not absorbed directly at the site of synthesis.

From the results of the present study it would seem that the change from soluble carbohydrate to modified starch, as represented by the change from sucrose to potato dextrin, is not the predominant factor in the improvement in nitrogen balances. The results obtained when 1% sulfasuxidine was incorporated in the rations are in agreement with the belief that the bacteria of the intestinal tract are in competition with the host organism for certain nutrients, and if they are changed in kind or amount the host may benefit.

Attention should be directed to the increases in fecal nitrogen excretion of the animals ingesting dextrin-containing rations. The animals of series 2 receiving corn dextrin excreted an average of 1.4 to 3.5 mg per day more fecal nitrogen than the same animals receiving the sucrose-containing rations. This was somewhat lower than the increase in nitrogen intake due to the change to the rations containing corn

dextrin. For the animals receiving potato dextrin, however, the increase in fecal nitrogen excretion was considerably higher than the increase in nitrogen intakes.

Booher, Behan and McMeans ('51) found that unmodified potato starch was relatively poorly digested when fed to rats, and stated that the apparent digestibility coefficients for protein varied directly with the digestibility of the starch component. Although no *in vivo* digestion trials were conducted in the present studies, it was apparent that the feces of the animals receiving the rations containing potato dextrin were bulkier and more numerous than those of the animals receiving either sucrose or corn dextrin. Harper, Katayama and Jelinek ('52) found that unmodified potato starch, autoclaved potato starch or potato dextrin incorporated in casein-containing rations fed to young rats induced greater excretion of certain amino acids in the feces than either sucrose or glucose. Further investigation is needed before speculating on the source of the extra fecal nitrogen observed when potato dextrin was fed in rations containing amino acids, in the present studies.

Dietrich, Monson and Elvehjem ('52) have shown that chicks and hyperthyroid rats fed purified rations gain at a greater rate if the rations contain corn dextrin than if sucrose is the carbohydrate. Moreover, if a 70% ethanol extract of dextrin is added to the sucrose-containing rations, the rates of gain approach those of the animals receiving dextrin. Further experiments will be required to determine whether the effect on nitrogen balance of a change in the carbohydrate from sucrose to corn dextrin is related to the unknown growth factor found in ethanol extracts of corn dextrin by these workers.

The present studies indicate that at these low levels of nitrogen intake, amino acid requirements for maintenance of nitrogen balance in the adult rat are significantly influenced by the carbohydrate moiety of the ration.

Since the preparation of this manuscript, a paper has appeared by Harper and Katayama ('53) demonstrating that

young rats fed rations containing 9% casein and cornstarch grew better than those receiving the same rations and sucrose. Substituting dextrin made from either cornstarch or potato starch supported growth equivalent to that obtained with cornstarch. The results of the present study, while differing at certain points, support the suggestion of Harper and Katayama that the type of carbohydrate in the ration may modify the requirement of the rat for amino acids, and extend the observations to adult rats and to rations containing free amino acids as well as intact proteins.

SUMMARY

The negative nitrogen balances of adult protein-depleted or undepleted rats fed rations containing low levels of amino acids (14.4 to 14.7 mg essential amino acid nitrogen per day) with sucrose as the carbohydrate were not significantly improved when the total nitrogen intake was increased by the addition of two levels of diammonium citrate. The negative nitrogen balances were significantly improved when the essential amino acid nitrogen intake was increased to 24.7 and 25.1 mg per day. Nitrogen losses of protein-depleted animals were less than those of undepleted animals at the same levels of total and essential amino acid nitrogen intake. When corn dextrin was substituted for sucrose in the rations, the animals achieved nitrogen equilibrium at levels of essential amino acid intake which resulted in large negative balances with the rations containing sucrose. The beneficial effects of feeding corn dextrin appeared to be due to the change in the carbohydrate and not to a gradual lowering of the negative nitrogen balances when animals received rations containing low levels of amino acids and sucrose for several weeks. Feeding 1% sulfasuxidine with corn dextrin brought about an additional improvement in the balances. When potato dextrin was substituted for sucrose there was a small but significant improvement in the nitrogen balances at the lower levels of amino acid intake but no difference at the higher levels.

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EFFECT OF 3-NITRO, 4-HYDROXYPHENYLARSONIC ACID AND ARSANILIC ACID ON SELENIUM POISONING IN THE RAT[1]

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(Received for publication March 23, 1953)

Arsenic, as sodium arsenite, is known to be effective in counteracting the toxic effects of selenium in certain animals (Moxon, '38, '41; Rhian and Moxon, '43; Moxon et al., '44). However, the toxicity of sodium arsenite makes its general use on farms and ranches in seleniferous areas somewhat impractical. Arsenic compounds of a lower order of toxicity might prove effective as a counteractant against selenium and be more suitable for use with livestock.

Arsanilic acid and 3-nitro, 4-hydroxyphenylarsonic acid are used as growth stimulants for some farm animals (Morehouse and Mayfield, '46; Morehouse, '49; Bird et al., '49; Carpenter, '51; Bird, '52). It was felt that if either of these two compounds would prevent the symptoms of selenium poisoning in animals, it might be useful as a supplement to feed in regions where this problem exists. The purpose of the studies reported here was to determine what effect, if any, these organic arsenicals might have on chronic selenium poisoning in the rat.

EXPERIMENTAL

Five series of rats were used during these investigations. In series I a preliminary study was made with the two organic

[1] Approved for publication by the Director of the South Dakota Agricultural Experiment Station as paper 294 of the Journal Series.

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arsenicals. Six groups of 4 female Sprague-Dawley albino rats were placed on experiment as indicated in table 1. Individual cages were used, and feed and water were allowed ad libitum. The percentage composition of the control (non-seleniferous) diets used for all series of rats was as follows: wheat (non-seleniferous), 82.5; casein (commercial), 10.0; dried brewers' yeast, 2.0; salts IV (Phillips and Hart, '35), 1.0; A and D feeding oil, 1.5; lard, 3.0. The composition of the toxic diet was the same as that of the control diet except that 28.4% of non-seleniferous wheat and 54.1% of seleniferous wheat (18.5 p.p.m. selenium) were used, giving a diet containing 10.0 p.p.m. of selenium. The protein levels, by analysis, of the two diets were as follows: control, 21.81%; toxic 21.25%. To insure optimum mixture of the arsenicals added to the basic diets, they were first dissolved in water, then sprinkled over the diets and mixed after drying. The level of arsenicals chosen for this initial study was the same as that found effective for sodium arsenite, based on arsenic content (Moxon, '38). However, since a reduced water consumption in the animals getting 3-nitro, 4-hydroxyphenylarsonic acid in the water was noted early in the experiment, the level of this compound was reduced by one-half for this group. Biweekly weight records were kept, and after 60 days the rats were sacrificed and their livers examined for extent of atrophy and cirrhosis.

The effect of 3-nitro, 4-hydroxyphenylarsonic acid administered through the drinking water at two higher levels was studied in series II. Four groups of 4 rats each (two male and two female) were placed on diets as described in table 1. The rats were handled in the same manner as in the first series, except that this experiment was terminated at 6 weeks because of the death of all the rats in the group receiving the toxic diet without added 3-nitro, 4-hydroxyphenylarsonic acid.

In series III, 3-nitro, 4-hydroxyphenylarsonic acid was supplied at still higher levels, this time mixed with the diet. Thirty-five female rats were divided into 5 groups of 7 rats

each and placed on diets as described in table 1. Here again the rats were handled as in the first series.

In series IV and V, arsanilic acid was fed through the diet at two levels much increased over the initial 5 p.p.m. level, as shown in table 1. The two series were handled as already described. Six male rats were used in each group for series IV, and three female and two male rats were used in each group for series V.

RESULTS AND DISCUSSION

The effectiveness of the two arsenicals used in these studies was judged on the basis of growth response, survival and protection against liver damage in the animal on a seleniferous diet. The results for all series of rats are given in table 1. In the first study (series I) neither 3-nitro, 4-hydroxyphenylarsonic acid nor arsanilic acid caused increased growth in the selenized animals. Both, however, afforded some protection against liver damage. It appeared that increased levels of these compounds might prove more effective, and the subsequent studies were made with this in mind.

When 3-nitro, 4-hydroxyphenylarsonic acid was fed at higher levels through the water (series II), increased protection against selenium damage was found. The arsenical caused reduced mortality, reduced liver damage and increased weight gains. The higher concentration of 3-nitro, 4-hydroxyphenylarsonic acid gave better protection against liver damage and death. As concerns average final weight, survival percentage must be taken into account, for deaths of the weaker group members raise the final weight average out of proportion with that of the other groups. However, weight data for this second experiment at 5 weeks, when none of the rats in either 3-nitro, 4-hydroxyphenylarsonic acid group had died, were as follows: 3-nitro, 4-hydroxyphenylarsonic acid at 5 p.p.m. arsenic — 164 gm; 3-nitro, 4-hydroxyphenylarsonic acid at 10 p.p.m. arsenic — 155 gm. Weight data therefore indicate no advantage of the higher level of the arsenic compound over the lower level in this series.

TABLE 1

Growth response, survival and protection against liver damage by selenium afforded by various levels of 3-nitro, 4-hydroxyphenylarsonic acid and of arsanilic acid

GROUP	DIET	AVERAGE BODY WEIGHT		PER CENT SURVIVAL	LIVER DAMAGE [1]
		Initial	Final		
		gm	gm		
	Series I — Low levels of 3-nitro, 4-hydroxyphenylarsonic acid and arsanilic acid given through the drinking water and diet				
a.	Control	98	225	100	—
b.	Toxic (10 p.p.m. selenium)	98	205	100	+++
c.	Toxic + arsanilic acid in diet at 5 p.p.m. arsenic	97	208	100	+++
d.	Toxic + arsanilic acid in H_2O at 5 p.p.m. arsenic	98	207	100	++
e.	Toxic + 3-nitro, 4-hydroxyphenylarsonic acid in diet at 5 p.p.m. arsenic	98	200	100	+
f.	Toxic + 3-nitro, 4-hydroxyphenylarsonic acid in H_2O at 2.5 p.p.m. arsenic	98	208	100	++
	Series II — Two levels of 3-nitro, 4-hydroxyphenylarsonic acid given through the drinking water				
a.	Control	69	225	100	—
b.	Toxic (10 p.p.m. selenium)	73	158	0	+++
c.	Toxic + 3-nitro, 4-hydroxyphenylarsonic acid in H_2O at 5 p.p.m. arsenic	72	195	50	++
d.	Toxic + 3-nitro, 4-hydroxyphenylarsonic acid in H_2O at 10 p.p.m. arsenic	71	180	100	+
	Series III — Two levels of 3-nitro, 4-hydroxyphenylarsonic acid given through the diet				
a.	Control	71	226	100	—
b.	Control + 3-nitro, 4-hydroxyphenylarsonic acid in diet at 25.6 p.p.m. arsenic	72	211	100	—
c.	Toxic + 3-nitro, 4-hydroxyphenylarsonic acid in diet at 14.2 p.p.m. arsenic	72	192	43	++
d.	Toxic + 3-nitro, 4-hydroxyphenylarsonic acid in diet at 25.6 p.p.m. arsenic	71	192	100	++
e.	Toxic (10 p.p.m. selenium)	72	130	30	+++
	Series IV — 0.009% arsanilic acid given through the diet				
a.	Control	74	315	100	—
b.	Toxic (10 p.p.m. selenium)	74	102	0	+++
c.	Control + arsanilic acid in diet at 31 p.p.m. arsenic	75	303	100	—
d.	Toxic + arsanilic acid in diet at 31 p.p.m. arsenic	75	251	67	++
	Series V — 0.025% arsanilic acid given through the diet				
a.	Control	77	248	100	—
b.	Toxic (10 p.p.m. selenium)	79	194	40	+++
c.	Control + arsanilic acid in diet at 86 p.p.m. arsenic	77	236	100	—
d.	Toxic + arsanilic acid in diet at 86 p.p.m. arsenic	82	213	100	—

[1] Liver damage designations represent the following:
— Normal liver.
+ Cirrhosis limited to small areas; liver size normal.
++ Cirrhosis more extensive; limited atrophy.
+++ Extensive atrophy and cirrhosis.

Protection against mortality could not be demonstrated in series I because none of the rats on the seleniferous diets died. The rats in this case were comparatively heavy when put on experiment (98 gm average). In series II the rats were started earlier (71 gm average weight) and mortality was high. This indicates a possible relationship between initial weight and chronic selenium toxicity.

The most practical method of feeding an arsenic compound to some farm animals is by mixing it with the ration. It has been observed in this laboratory that sodium arsenite must be mixed at a higher level in feed than when fed in the water, to give the same degree of protection. In series III, IV and V, therefore, the arsenicals were mixed in the feeds at fairly high levels. With 3-nitro, 4-hydroxyphenylarsonic acid, liver damage protection was not complete even at the highest level used, and only at this level was the number of deaths significantly reduced. It is evident that the weight gains at the two levels of this arsenical were not much different, although the mortality in the 14.2 p.p.m. arsenic group did raise the average weights late in the experiment, to account for some of the final weight similarity.

Preliminary work with arsanilic acid fed at a 5 p.p.m. arsenic level (0.0015% arsanilic acid) indicated that increased levels might better counteract selenium toxicity. In series IV it was found that the administration of a 31 p.p.m. arsenic level (0.009% arsanilic acid) did result in greater growth response, but gave only minor protection against liver damage and only partial prevention of death. Arsanilic acid has been found to be well tolerated by chicks and turkeys at a 0.03% level (Frost, '52). On the basis of this work, arsanilic acid was fed in series V at a 0.025% level, or 86 p.p.m. arsenic. Feed consumption appeared normal and the rats showed good tolerance to the high arsenical level. The arsanilic acid proved effective at this level on the basis of either survival, liver protection or growth stimulation.

++ Cirrhosis more extensive; limited atrophy.
+++ Extensive atrophy and cirrhosis.

In series III, IV and V the arsenicals were fed rats on a selenium-free diet. No stimulation in growth was found under the conditions of these experiments. In fact, the compounds when fed at the higher levels appeared to depress growth slightly.

In none of the experiments discussed here did either arsenical give complete protection against selenium poisoning, even at the highest levels used. However, both arsanilic acid and 3-nitro, 4-hydroxyphenylarsonic acid gave partial protection against the effects of selenium, the degree of protection depending upon how much of the arsenical was used. In view of the fact that these compounds may prove less hazardous to use on the farm, where their administration can be better controlled, and also in view of their reported stimulatory effect on the growth of some farm animals, arsanilic acid and 3-nitro, 4-hydroxyphenylarsonic acid should be further tested as selenium poisoning preventatives in hogs and chickens.

SUMMARY

1. Arsanilic acid and 3-nitro, 4-hydroxyphenylarsonic acid were found to give partial protection against chronic selenium poisoning in rats. The degree of protection increased as the levels of the two arsenicals increased. However, even at the highest levels used (arsanilic acid — 0.025%; 3-nitro, 4-hydroxyphenylarsonic acid — 0.009%) complete protection was not observed.

2. Levels of 0.009% 3-nitro, 4-hydroxyphenylarsonic acid and 0.009 and 0.025% arsanilic acid gave no growth response with the non-seleniferous control diets used in these experiments.

ACKNOWLEDGMENTS

The authors express their appreciation to Dr. Douglas V. Frost, Abbott Laboratories, for supplying the arsanilic and the 3-nitro, 4-hydroxyphenylarsonic acid used in this work, and for his valuable suggestions during the course of the work.

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PROCEEDINGS OF THE SEVENTEENTH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

CONRAD HILTON HOTEL, CHICAGO, ILLINOIS
APRIL 6–10, 1953

COUNCIL MEETINGS

Council meetings were held in the Conrad Hilton Hotel on Sunday, April 5, and Monday, April 6. Formal actions of the Council are reported in the following minutes of the business meetings.

SCIENTIFIC SESSIONS

The scientific program of the Institute consisted of 9 half-day sessions at which 93 papers were presented and one half-day session devoted to a symposium on "The Metabolic Significance of the B Vitamins." The symposium was held on Wednesday afternoon, April 8, and was unusually well-presented and well-attended. Six papers were read by title.

BUSINESS MEETINGS

Two business meetings were held: one at 4:15 P.M. Tuesday, April 7, and one at 4:00 P.M. Thursday, April 9. The following items were considered:

Tuesday, April 7, 4:15 P.M. The meeting was called to order by President Paul L. Day. The minutes of last year's business meetings, as published in the September, 1952, issue of the *Journal of Nutrition,* were approved.

The President appointed Dr. D. V. Frost and Dr. L. R. Richardson to serve as a Tellers' Committee for the election

of officers. The Secretary transmitted the ballots to the Tellers' Committee for counting.

The Council's recommendations on nominees for election to membership in the American Institute of Nutrition were presented. It was voted to approve the Council's recommendations. The following were elected to membership:

Damon Catron
J. S. Dinning
R. M. Forbes
L. E. Harris
R. A. Harte
R. L. Jackson
B. M. Kagan
T. D. Luckey
F. H. Mattson
M. B. Patton
B. L. O'Dell
Homer Patrick
Hugh Sinclair
S. E. Snyderman
R. L. Squibb
A. E. Teeri
M. G. Wohl

The report of the Treasurer, Dr. N. B. Guerrant, was submitted. The Auditing Committee, consisting of Dr. Alex Black and Dr. Cyrus E. French, reported that receipts and disbursements were properly recorded in the Treasurer's books and that his report as of April 1, 1953, was substantiated by the record. The continuation of the $1.00 annual dues for next year, as recommended by the Treasurer and approved by the Council, was approved.

The Secretary, Dr. J. M. Orten, reported that as of April 1, 1953, the membership of the Institute includes 362 active members and 31 retired members, totalling 393. The following 5 members died during 1952–53: Dr. Nellie Halliday, Dr. E. B. Hart, Dr. P. C. Jeans, Dr. H. A. Mattill, and Dr. W. T. Salter.

The Secretary reported the results of the questionnaire regarding holding all Federation meetings from 1956 to 1960 in Atlantic City. Approximately 50% of the members were opposed. Similar results were reported by other societies of the Federation. In view of this fact, the Federation Board voted to continue the present plan of alternating between Atlantic City and other cities.

The annual report of the Editor of the *Journal of Nutrition* was presented by Dr. George R. Cowgill. This report follows:

Annual Report of the Editor of the Journal of Nutrition

This annual report covers the volumes of the Journal of Nutrition published during 1952 as well as general matters arising since the last annual meeting of the American Institute of Nutrition in New York City, April 15, 1952.

Papers published and related data

Three volumes — numbers 46, 47 and 48 — were published during 1952. Each began with a biography. In addition, these three volumes contained 48, 50 and 44 articles, respectively, giving a total of 142 for the year, compared with 144 for the previous year. The average number of pages per article proved to be 12.6; the corresponding figure for the previous year was 12.5 pages. During 1952, subscribers received a total of 1,744 pages compared with 1,841 pages for the previous year. During the year, 185 papers were submitted of which 37, or 20%, were rejected. The corresponding figures for the previous year are 197 submitted and 39, or 19.8%, rejected.

Picture on front cover

In continuation of the policy adopted at the 1949 meeting of the editorial board in Detroit, the first issue of each volume carried a new picture in the oval on the front cover. A larger picture was used as frontispiece, accompanied by a short biography. The pictures used in 1952 were those of Mulder, Kellner and Rubner.

Personnel of editorial board

At the last annual meeting three members retired, having served their terms of 4 years. They were R. H. Barnes, Paul L. Day and H. H. Mitchell. The new members elected to replace them were James B. Allison, Carl A. Baumann and Leo C. Norris.

Editorial problems

These continue to be much the same as those encountered in previous years. We have continued the policy of having a meeting of the editorial board sometime during the Federation meetings. At this time our editorial problems are reviewed, and any matter pertaining to the welfare of the Journal and its management considered. The policies followed by the editor are those which the editorial board has discussed and adopted.

I wish to express my deep appreciation of the services rendered by the individual members of the editorial board. I might add that in our joint efforts we always try to avoid mere criticism for its own sake, but instead endeavor to make our comments constructive and in the interest of a better paper and a better publication as we see each problem presented. Any examination of our office correspondence will show that most of it deals with ways in which papers might be improved through changes of one kind or another, with some saving in space required and in cost of printing.

Our thanks are also due to the staff of The Wistar Institute. They have always cooperated in the best possible manner in solving special printing problems.

A motion, as approved by the Council, was unanimously passed authorizing the Editor to make available limited space in the *Journal of Nutrition* for resolutions and formal actions of the Food and Nutrition Board of the National Research Council in matters relating to basic problems in nutrition.

A motion was passed expressing appreciation to Dr. Cowgill for his excellent administration of the affairs of the Journal during the past year.

The proposed change in the constitution and by-laws to include the Past President as a member of the Council was discussed. The Secretary stated that the rules for amending the constitution had been fully complied with in this case. The amendment was unanimously approved. The affected portions of the constitution and by-laws will hereafter be stated as follows:

Constitution: Section 3 — "The management . . . in a Council consisting of the President, Vice-President, Past President . . ."

By-Laws: Article III, Section 2 — Council — "The officers so elected, the Past President, and three additional members . . ."

Reports from the Standing Committees were next presented.

Dr. W. C. Russell, representative to the Division of Biology and Agriculture and to the Food and Nutrition Board of the

National Research Council, submitted a report of the actions of these two groups during the year. A summary of Dr. Russell's report is on file in the Secretary's office.

The representatives to the Joint Committee on Nomenclature with the Society of Biological Chemists, Dr. C. A. Elvehjem and Dr. E. M. Nelson, reported that one question on nomenclature had been referred to their committee and that the matter was under consideration.

The Secretary read a brief report from Dr. Herbert Pollack, Chairman of the Committee on the Registry of Pathology of Nutritional Diseases, stating that the affairs of the Registry were in good order. A summary of the work of the Registry during the past year appeared in the February, 1953, issue of the *Journal of Nutrition,* as authorized by the Council.

A brief report on recent actions of the Federation Board was presented by the Secretary. A new Federation rule on the transfer of papers at the annual meetings was discussed. The new rule states that transfer requests may be rejected by a society secretary if the requestor has already submitted a paper to the society in question.

Federation Board actions in rejecting a proposed "prepublication" of abstracts and in changing the form of the printed program of the annual meeting were also discussed.

Recent actions of the Council of the Institute were briefly discussed. These included affiliation with the American Association for the Advancement of Science, as approved by the Council, the rewording of the "Rules of Awards" administered by the Institute, and the continuing of a Joint Smoker with the Society of Biological Chemists at the annual meetings.

The meeting was adjourned at 5:10 P.M.

Thursday, April 9, 4:00 P.M. The meeting was called to order by President Paul L. Day.

Dr. Helen Oldham presented a report on the International Institute of Food and Nutrition.

The Tellers' Committee reported the results of their count of the ballots. A total of 217 ballots was cast. The following officers were elected for the year beginning July 1, 1953:

President:
Conrad A. Elvehjem

Vice-President:
George R. Cowgill

Treasurer:
O. L. Kline

Councillor:
Harry D. Kruse

Associate Editors:
E. W. Crampton
Ruben W. Engel
Gladys A. Emerson

Suggestions for membership on the Nominating Committee:
Fourteen members received 10 or more votes each.

President Day reminded members of the Institute of the invitation to participate in the 19th International Physiological Congress to be held in Montreal, August 31 to September 4. He also commented briefly on the International Union of Nutritional Sciences and on the meeting sponsored by the British Society of Nutrition in Edinburgh, Scotland, commemorating the 200th anniversary of Lind's work on scurvy.

President Day announced that next year's meeting would be held in Atlantic City and that the 1955 meeting would be held on the West Coast, either in Los Angeles or San Francisco.

The meeting was turned over to the new President, Dr. Conrad A. Elvehjem, and was adjourned at 5:00 P.M.

ANNUAL DINNER AND PRESENTATION OF AWARDS

The Annual Dinner of the American Institute of Nutrition was held on Wednesday evening, April 8, in the Conrad Hilton Hotel and was attended by some 200 members and guests. The program consisted of the introduction of new members, the presentation of awards, and a few informal remarks by President Paul L. Day.

The Borden Award in Nutrition was presented to Dr. Harold H. Williams of Cornell University at Ithaca for his research contributions on the vitamin components of human milk and on nutritional and biochemical changes during lactation. The achievements of the medalist were described by Dr. Harry

J. Deuel of the University of Southern California. Dr. Williams responded with a brief resume of his work and with descriptions of his experiences during his training as a nutritional biochemist.

The Osborne and Mendel Award was presented to Dr. Vincent du Vigneaud of the Medical College of Cornell University for his investigations on the chemical structure, metabolic interrelations, and nutritional significance of certain essential dietary constituents. Dr. Joseph H. Roe of George Washington University introduced the recipient and commented on his signal qualifications for the honor being bestowed upon him. Dr. du Vigneaud then reminisced about his work in the field and particularly of his personal contacts with Professor Lafayette B. Mendel, one of those for whom the Award is jointly named.

COMMITTEES FOR 1953–1954

President Paul L. Day appointed the following committees for the year beginning July 1, 1953:

Nominating Committee

L. A. Maynard, Chairman
H. R. Bird
W. J. Darby
W. H. Griffith
A. H. Smith

Committee on Registry of Pathology of Nutritional Diseases

Herbert Pollack, Chairman
O. A. Bessey
W. H. Sebrell, Jr.
E. L. Sevringhaus
C. L. Pirani, Secretary

Consultants

P. R. Cannon
Paul Klemperer
B. S. Wolbach

Representatives to the Joint Committee on Nomenclature

C. G. King
E. M. Nelson

Representative to the Division of Biology and Agriculture and to the Food and Nutrition Board, National Research Council

W. C. Russell

Respectfully submitted,

JAMES M. ORTEN, *Secretary*
American Institute of Nutrition

BORDEN AWARD IN NUTRITION

Nominations are solicited for the 1954 Award and a gold medal made available by the Borden Company Foundation, Inc. The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the Jury of Award may recommend that it will be given for important contributions over an extended period of time. The award may be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

The formal presentation will be made at the annual meeting of the Institute in the spring of 1954. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 1, 1954. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

Chairman, Nominating Committee:

DR. JOHN B. BROWN
Institute of Nutrition and Food Technology
Ohio State University
Columbus, Ohio

OSBORNE AND MENDEL AWARD

Nominations are invited for the Osborne and Mendel Award of $1000.00 established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the Award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

Nominations may be made by anyone. Nominations for the 1954 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 1, 1954.

Chairman, Nominating Committee:

DR. H. E. ROBINSON

Research Laboratories
Swift and Company — Union Stock Yards
Chicago 9, Illinois